THE USE OF POLARIZED LIGHT IN THE STUDY OF MYELIN DEGENERATION

II. THE DEGENERATION OF MYELINATED NERVES IN AVITAMINOSIS A IN THE WHITE RAT

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FOUR FIGURES

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INTRODUCTION

Early in 1927 symptoms indicative of nervous disorders were first noted in certain groups of rats in our colony which were being used in vitamin A assays. The highest incidence of the symptoms in question was in groups receiving the lower levels of vitamin A supplement after being completely depleted of vitamin A. Animals which were completely depleted of vitamin A were characterized by extreme xerophthalmia, emaciation, great weakness and early death. After from 45 to 50 days on a vitamin A free diet, plus low levels of vitamin A supplement after depletion, these rats showed symptoms not previously described as associated with avitaminosis A.

The early symptoms in these animals are characterized by a peculiar weaving, unsteady gait in the hind limbs. There is a definite lack of coordination rather than an actual paralysis in early stages. Frequently in moving about the limb or limbs are abducted to a degree greater than normal. There soon develops a relaxed condition of the leg; the limb being partly extended at all times while walking. The animal seems unable completely to flex the knee and hip joint. If the rat is held up in a vertical position the affected leg is allowed to

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hang in a relaxed, extended posture while the unaffected one is drawn up close to the trunk. The condition is usually unilateral and in cases of bilateral involvement the symptoms are always more pronounced in one leg. There is, however, no predilection for either side. We have seldom found the condition affecting the front legs. We believe this is due to the fact that the rats succumb before the progress of the disease has reached them rather than any limitation of the disease itself to the hind legs.

Fig. 1 Rat 724. Diagnosis ++++. Weight 104 gm. Weight of positive control in same group 214 gm.

This rat was fed a vitamin A free diet for 35 days. At that time it had stopped gaining weight and had xerophthalmia. It was then given 30 mg. butter fat per day. At the end of 10 days it showed symptoms of nervous disorder. These progressed steadily until the animal was killed after 4 weeks on the supplemented diet (30 mg. of butter fat is less than a unit dose). (Table 1.)

Note: roughened fur, swollen conjunctiva, abduction of leg, flexion of toes, relaxed position of posterior half of the body. Not apparent in the picture: flaccid and atrophic muscles of the thigh and leg.

With the progress of the disease the ability to use the affected parts diminishes. The rat walks on its heels with little or no use of the digits. The muscles of the thigh and leg become flaccid and then atrophied. The digits are carried always in the flexed position. We have never noted a convulsive or spastic condition. In moving about the leg is frequently abducted to such a degree that the animal walks on the side of the heel rather than on the sole of the foot. The body is carried low, the belly almost or quite touching the floor. The legs are spread apart and extended. The muscles of the tail are often affected and sometimes atrophy. In a few cases the tail muscles on one side only are affected and

the tail is then curved away from the affected side. A painful stimulation of the tail or toes brings only a feeble response or none at all. The animals frequently chew their own tails or feet. In a few cases individuals have chewed off the whole tail or a whole foot up to the ankle joint. The final result of the disease is an irreparable atrophy of the

Fig. 2 Section of a normal sciatic nerve of white rat. This animal had been on a complete diet. Photographed in polarized light, between crossed nicols, at a magnification of 1200.

Note: The dark lines are axis cylinders, the light areas are myelin sheaths. Normal node of Ranvier in lower right.

muscles of the thigh and leg. Animals which have suffered such atrophy do not recover control or replace muscle fibers even though a complete diet brings about complete recovery from all other symptoms of avitaminosis A.

We suspected that the spinal nerves to the parts affected were involved in the disease and were degenerating due to

some unknown effect of the vitamin deficiency. It was in an attempt to describe the effects of avitaminosis A on the peripheral nerves of the rats that this study was begun.

In the early stages of the work the sciatic and femoral nerves and spinal cords were removed and some of them fixed

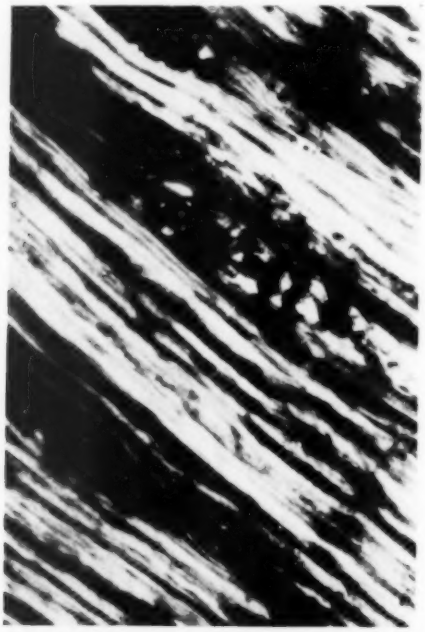

Fig. 3 Diagnosis ++++. This rat (701) received the same diet as 724. It was in about the same physical condition except that the xerophthalmia had improved somewhat more than in the other rat. Not quite so many fibers are involved but those affected are in a later stage of degeneration. Compare the greatly swollen fibers with those in figure 7 of preceding paper.

Photographed in polarized light between crossed nicols at a magnification of 1200.

in potassium bichromate and further prepared by the Marchi method. Others were fixed in 10 per cent formalin and later frozen sections of them were stained with Sudan III or Scharlach R. We found, as had Duncan ('31) and Prickett ('34), that in the Marchi preparations blackened droplets

were visible in normal controls as well as in our diseased animals. Likewise the fat stains, Scharlach R and Sudan III were unsatisfactory since they depend on relative intensity of color and since this varies with the temperature, thickness of section, concentration of stain, degree of degeneration, and

Fig. 4 Diagnosis ++++. Rat 755. This rat was depleted in 35 days and then received 30 mg. of butter fat per day for 3 weeks. It showed nervous symptoms for 1 week before it was killed. The appearance of the fibers resembles those in plate 3 but more fibers in the early stages of degeneration are present. One in the center of the field is in a late fragmenting stage.

Photographed in polarized light between crossed nicols at a magnification of 1200.

length of time stained. While we were satisfied that the nerves were affected we could not demonstrate it with certainty in early stages by any of the three methods named.

In searching the literature for a more delicate technic the statement in Lee ('21) by Cramer and Lee concerning the

reactions of various lipids was noted. Cramer there states that the true fats, or triglycerides, are isotropic while the phospholipids are doubly refractive, or anisotropic. It is therefore possible to distinguish these two groups of substances by the use of the polarizing microscope.

Following this lead and the other facts noted in the first paper of this series we applied this test for the presence of the degeneration products of myelin (triglycerides) in the nerves from our rats. Finding that we could observe very slight changes from the normal we proceeded to establish the technic for the study of degeneration by using it in a series of animals in which experimentally produced Wallerian degeneration was present. This work constituted the subject matter of the first paper of this series (Setterfield and Sutton, '35).

MATERIALS AND METHODS

Feeling that our experience with the new technic was too limited to include the central nervous system in our observations, we restricted our material to the spinal nerves related to the affected parts. These were prepared and studied as described in the preceding paper. We studied nerves from a total of eighty-five rats on various levels of vitamin A supplement as well as normal controls.

It was found that among forty-eight rats on an extremely low level of vitamin A supplement after depletion (30 mg. butter fat per day) 97.9 per cent of them developed nervous symptoms and soon died. Of these we observed nerves from twenty-one. Nine of them were diagnosed as +++[1] while

[1] The signs ⊥, +, ++, +++, and ++++ are used to describe the severity of degeneration and roughly the extent of involvement of the nerve fibers in a nerve. ⊥ refers to a condition where there is only a slight swelling of a few fibers and no isotropic material. + refers to the stage when one or two fibers in the section are greatly and irregularly swollen and are beginning to lose their birefringence. ++ is a stage where approximately every third fiber is degenerating, greatly swollen and showing extensive isotropic masses.

In the +++ stage about two out of three fibers are badly degenerating. In the ++++ stage every fiber is affected more or less, ranging from early degeneration in some to extreme fragmentation and isotropic condition in others.

All these stages are arbitrary and not exact. Their value is relative and they are simply useful terms in describing the course of degeneration.

twelve were ++++. It was subsequently found that this was also true when the rat received 1 mg. cod liver oil or 0.4 gamma of carotene per day after depletion (table 3). Among twenty rats on an adequate[2] vitamin A supplement (60 mg. of butter fat per day) after depletion, none of the animals developed symptoms (table 1). However, later studies of the time of appearance of degeneration compared with the time symptoms developed showed that degeneration had begun but had not proceeded far enough to produce external signs. Duncan ('30) has stated that degenerating fibers are always found in the peripheral nerves of the normal rat and that in rats low in vitamin A the rate of degeneration is

TABLE 1

The occurrence of nervous symptoms in rats receiving a vitamin A deficient diet, supplemented with varying levels of butterfat after cessation of growth and appearance of zerophthalmia

NUMBER OF RATS	LEVEL OF BUTTERFAT SUPPLEMENT	PER CENT OF RATS SHOWING NERVOUS SYMPTOMS	MICROSCOPICAL DIAGNOSIS
20	60 mg. daily	00	No microscopic diagnosis
48	30 mg. daily	97.9	The femoral and sciatic nerves from 21 of these rats were examined microscopically 9 showed +++ degeneration 12 showed ++++ degeneration

increased. However, the rat in this study that showed the greatest number of degenerating fibers was vitamin A deficient and free from infection, indicating that the degeneration was not due to infection. He has estimated that less than 1 per cent of nerve fibers in the sciatic nerve of a normal rat may be degenerating at any one time. The rate of degeneration increases with age. However this percentage represents less than one fiber in a whole sciatic nerve. Degeneration in our rats at the time under discussion (⊥ or +) has gone much further, there being from one to four fibers

[2] By an adequate supplement of vitamin A is meant an amount per day sufficient to produce as great an average increase in weight per day as is shown by normal stock rats of the same age which have not been depleted and are on a complete diet.

in an average high power field definitely degenerating at this time. We have not made total counts to show the exact extent to which degeneration may go before external signs of the loss are visible.

TABLE 2

The progress of nerve degeneration in avitaminosis A. (Basal diet —A, 0.5 gm. yeast 2.6 units of vitamin D (viosterol) daily)

RAT NO.	DAYS ON DEFICIENT DIET	DURATION OF XEROPHTHALMIA	CHANGE IN WEIGHT FROM MAXIMUM	MICROSCOPIC DIAGNOSIS	
				Femoral	Sciatic
C802	28	No xerophthalmia	Still gaining	Normal	⊥
C805	28	No xerophthalmia	Still gaining	Normal	⊥
C804	31	No xerophthalmia	Still gaining	Normal to ⊥	Normal to ⊥
C807	31	No xerophthalmia	Still gaining	Normal	Normal to ⊥
C803	34	Incipient xerophthalmia	Still gaining	+	+
C806	34	Incipient xerophthalmia	Still gaining	+	+
C812	34	Incipient xerophthalmia	Still gaining	+	+
C813	34	Incipient xerophthalmia	Still gaining	+	+
C668	39	3 days	Still gaining	+	+
C673	39	3 days	Still gaining	+	+
C667	43	7 days	— 5 gm.	+	++
C675	43	7 days	— 9 gm.	++	++
C671	50	7 days	— 12 gm.	+++	+
C670	56	10 days	— 34 gm.	+++ (wobbly gait in rear limbs)	+++
C672	56	10 days	— 16 gm.	+++ (impaired use of right foot)	+++
C674	60	20 days	— 25 gm.	++++ (impaired use of left foot)	++++
C666	72	26 days	— 21 gm.	+++ (wobbly gait, poor coordination)	++++

The study referred to above, summarized in table 2, was the diagnosing of nerves from rats on a deficient diet beginning 8 days before the appearance of xerophthalmia or

cessation of growth. In these the diagnoses were ⊥ and + of degeneration even though there were no external signs whatever of vitamin deficiency. By the time the rats began to lose weight the amount of degeneration had increased to ++

TABLE 3

Progress of degeneration is arrested by adequate dosages of vitamin A supplements administered after xerophthalmia develops. (Unless otherwise designated, all rats were killed after 8 weeks of supplement feeding.) (Basal diet —A_{11} 0.5 irradiated yeast)

RAT NO.	SUPPLEMENT	CHANGE IN WEIGHT DURING SUPPLEMENT FEEDING	DURATION OF NERVOUS SYMPTOMS BEFORE KILLING OR DEATH	MICROSCOPIC DIAGNOSIS (FEMORAL AND SCIATIC NERVES)
C688	30 mg. B.F.	— 5[1] (42 days)	7 days	+++
C676	30 mg. B.F.	— 20[1] (29 days)	8 days	++++
C694	30 mg. B.F.	— 16[1] (43 days)	21 days	+++
C682	30 mg. B.F.	— 2	21 days	+++
C690	1.0 mg. C.L.O.	— 6[1] (46 days)	14 days	+++
C677	1.0 mg. C.L.O.	+ 16	21 days	+++
C695	1.0 mg. C.L.O.	— 10[1] (41 days)	13 days	+++
C686	1.0 mg. C.L.O.	+ 28	28 days	++
C692	0.4r carotene	+ 34	14 days	++
C678	3 weeks 0.8r	+ 51	28 days	+++
C696	Carotene 5 weeks	+ 25	42 days	+++
C684	Carotene 5 weeks	+ 54	Kept for demonstration	
C679	90 mg. B.F.	+ 56	No symptoms	+
C685	90 mg. B.F.	+ 56	No symptoms	⊥
C680	3.0 mg. C.L.O.	+ 50	No symptoms	+
C683	3.0 mg. C.L.O.	+ 66	No symptoms	⊥
C681	1.2r carotene	+ 52	No symptoms	++
C687	1.2r carotene	+ 75	No symptoms	⊥
C699	1.2r carotene	+ 64	Kept for demonstration	

[1] Rat died before the end of the experiment.

and from that time progressed to +++ during the first 7 days of xerophthalmia and to ++++ by 20 days of xerophthalmia. By the time degeneration had reached +++ in the sciatic the animal's locomotion was greatly impaired and both sensibility and motor control greatly diminished (table 2).

Progress of degeneration can be arrested by adding adequate amounts of vitamin A to the diet of the depleted animals. In rats receiving only 30 mg. of butter fat per day the degeneration progressed steadily to ++++ and the early death of the animals. However if, when xerophthalmia appears (i.e., at least 6 days after degeneration begins) the diet is supplemented by 90 mg. of butter fat per day the degeneration remains at the ⊥ or -+ stage which is always present when xerophthalmia appears. These facts are summarized in table 3.

The observations cited above demonstrate the presence of degeneration in amounts and at times not observable by any other technic.

All the diagnoses in the eighty-five rats studied were made objectively, the observer in no case being aware of the physical condition of the animal from which the nerves were taken. More detailed discussion of the nutritional aspects of these vitamin A studies has been published under appropriate title (Sutton, Setterfield and Krauss, '34). Our purpose in this paper is to describe the application of the polarized light technique to the study of very early and very small amounts of degeneration in spinal nerves.

CONCLUSIONS

1. Polarized light has been used to study qualitatively, quantitatively and chronologically the progress of myelin degeneration in rats on diets deficient in vitamin A and diets supplemented by various amounts of vitamin A.

2. Degeneration in the sciatic and femoral nerves begins from 3 to 6 days (at least) before there is any external sign of avitaminosis A.

3. Between one-fourth and one-third of the fibers are in the process of degeneration before there is any external sign of loss of sensibility or motor control in rats suffering from avitaminosis A.

4. Adequate dosages of vitamin A administered after depletion arrested the progress of degeneration.

LITERATURE CITED

DUNCAN, DONALD 1930 The incidence of secondary (Wallerian) degeneration in normal mammals compared to that in certain experimental and diseased conditions. J. Comp. Neur., vol. 51, pp. 197–228.

———— 1931 The Marchi method: A discussion of some sources of error and the value of this method for studying primary changes in the myelin sheath. Arch. Neur. and Psych., vol. 25, pp. 327–355.

LEE, ARTHUR BOLLES 1921 Microscopists' Vade Mecum, p. 351.

PRICKETT, C. O. 1934 The effect of a deficiency of vitamin B upon the central and peripheral nervous systems of the rat. Am. J. Physiol., vol. 107, pp. 459–470.

SETTERFIELD, H. E., AND T. S. SUTTON 1935 The use of polarized light in the study of myelin degeneration. I. The appearance of progress of degeneration after transection of the sciatic nerve of the white rat. Anat. Rec., vol. 61, pp. 397–411.

SUTTON, T. S., H. E. SETTERFIELD AND W. E. KRAUSS 1934 Nerve degeneration associated with avitaminosis A in the white rat. Ohio Agri. Exp. Sta., Bull. 545,

THE EFFECT OF PROTEIN AND AMINO ACID METABOLISM ON THE UREA AND XYLOSE CLEARANCE

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THREE CHARTS

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Investigators who have studied the kidney under suitably controlled conditions have frequently remarked on the variations in renal function that seem to be of a physiological nature. Most noteworthy in this connection is the influence of the maintenance diet, as well as the immediate influence of the ingestion of food. Addis and Drury ('23) observed that the rate of excretion of urea relative to the blood urea is increased in man after feeding milk or glutamic acid. Jolliffe, Shannon and Smith ('32) have observed that urea, xylose and creatinine clearances in the dog are not only elevated by the feeding of meat, but are greater when observed in the post-absorptive condition in dogs which are maintained on a meat diet than in the same dogs maintained on a low protein diet. These investigators have concluded that the functional changes are related to changes in the rate of glomerular filtration.

The experiments reported in the present paper are intended to examine to what extent these variations in activity are related to meat, as compared with other proteins; to what extent they are dependent on digestion and absorption on the one hand or metabolism on the other; and to what extent they can be correlated with any specific phase of protein metabolism.

METHODS

Female dogs were used throughout. Urine was obtained by catheter with care to insure complete emptying of the bladder, and blood was drawn from the external jugular vein. Urea was determined by the gasometric method of Van Slyke ('27), and xylose as described by Jolliffe, Shannon and Smith ('32).

The basal diet consisted of 100 gm. of cracker meal, 30 gm. of cane sugar, 30 gm. of butter, 10 cc. of cod liver oil, 5 gm. of dry irradiated yeast and 0.6 gm. of Cowgill's salt mixture, moistened with warm water and well mixed. The dogs were fed at about 5 P.M. and, unless otherwise stated, the experiments were performed 16 to 18 hours after feeding.

RESULTS

It is evident that results obtained on different animals can be compared only with great uncertainty, and for this reason we have made our observations as far as possible on the same animals under a variety of conditions. It has been impossible to present in tabular form the extensive data of our experiments, and for this reason representative experiments have been summarized graphically in the appended charts. These charts are uniform in having the xylose clearance (upper line) and the urea clearance (lower line) plotted against time expressed in units of 100 minutes. The data at the bottom of the charts state the types of diet and the time which the dogs had been maintained thereon. The figures above these data state the average ratio: urea clearance/xylose clearance. Every experiment, unless otherwise noted, was begun with a series of three control observations made in the post-absorptive condition at least 16 hours after the last meal. The dog was then fed or injected, as the case might be, and the clearances were observed for some time thereafter. The dotted lines indicate periods in which no urine collections were made, and the figures written beside arrows pointing to these lines indicate instances where there was a lapse of time that could not be expressed on the charts. The urine collection periods averaged 30 minutes each and the

urine flows ranged from 1 to 6 cc. per minute, with the exception of experiment 78 in which they ranged from 0.59 to 0.93 cc. All of these are above the augmentation limit.

Experiment 32 (chart 1) is a series of control observations on dog 7 made in the post-absorptive state after 14 days on a basal diet. The average data (urea clearance/xylose clearance = X) for five consecutive periods are $13.9/19.7 = 0.70$. Five days later (experiment 33), this dog was again observed in the post-absorptive state: $16.5/20.5 = 0.80$. The dog was

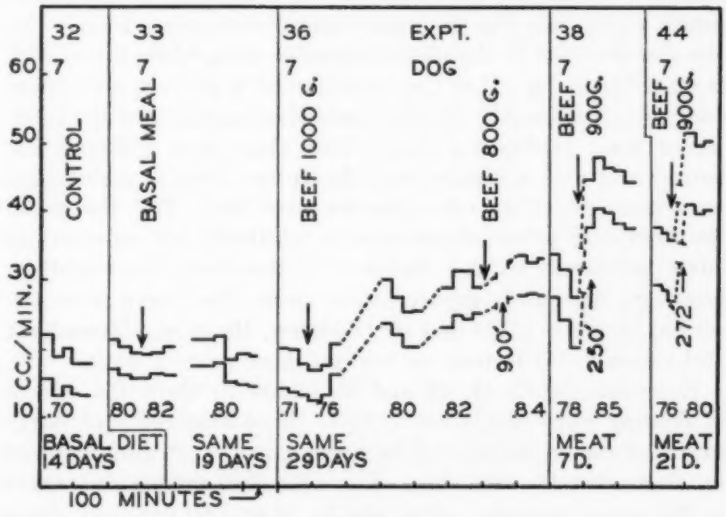

Chart 1

then fed a basal meal without any significant change in the average clearance levels of either urea or xylose. Ten days later (experiment 36), this experiment was repeated, substituting 1000 gm. of raw chopped beef for the basal meal, whereupon the clearances rose from a control level: $13.9/19.6 = 0.71$, to: $24.7/30.3 = 0.82$. Another meal of 800 gm. of raw beef raised the clearances to: $28.1/33.4 = 0.84$. After a week on a meat diet consisting of 900 gm. of raw chopped beef daily, both clearances (experiment 38) remained at an

elevated level in the post-absorptive condition (24.3/31.2 = 0.78), and now a further feeding of meat increased them again: 39.1/46.3 = 0.85. This is, respectively, 2.81 and 2.36 times the post-absorptive basal-diet level obtaining a week earlier, before the first meat meal. After 2 more weeks on a meat diet there was a still further increase in the post-absorptive clearances: 28.3/37.2 = 0.76; and after feeding meat these clearances again rose in the usual manner to: 40.6/51.1 = 0.80, or to 2.92 and 2.60 times the original basal level. These results confirm the observations of Shannon, Jolliffe and Smith ('32) that the urea and xylose clearances tend to be low and constant in the post-absorptive state while the animal is on a basal diet; that the ingestion of a protein-poor meal does not significantly elevate these clearances; that the feeding of meat produces a rise in both clearances within a few hours; and that a higher post-absorptive level is maintained on a meat diet than on a protein-poor diet. The change in the urea and xylose clearances is relatively not so great in our experiments as was observed by the above investigators, however, but much greater than those that have been observed by Cope ('33) and by Goldring, Razinsky, Greenblatt and Cohen ('34) in man on low and high protein diets.

Experiments 40, 41, 42 and 45 (chart 2) show the effects of feeding pure casein for 7 days. Two hundred and thirty grams of casein, suspended in warm water, were administered by stomach tube each day. The urea and xylose clearances in the post-absorptive state rose to 74 and 60 per cent above the basal level and increased to 109 and 89 per cent above the basal level after a casein meal. These changes are somewhat less than were obtained with meat, but leave no doubt that casein acts qualitatively in the same manner as meat.

On the day previous to experiment 41 and after the control observations of experiment 40 had been made, the dog refused to eat and there was a significant drop in the level of the urea and xylose clearances, but these were returned toward the normal post-absorptive level by feeding the basal meal (see chart). It appears that the post-absorptive level obtained on a protein-poor maintenance diet is a basal level

only in the sense that it is relatively constant and not in the sense that it is minimum.

To exclude the participation of the gastro-intestinal tract in these phenomena, metabolism of endogenous protein was stimulated by thyroxin. Experiment 46 (chart 2) shows that thyroxin in doses of 10 mg. has no appreciable effect on renal activity the first day, but experiment 47 shows that a sharp rise occurs on the second day; after three additional 10 mg.

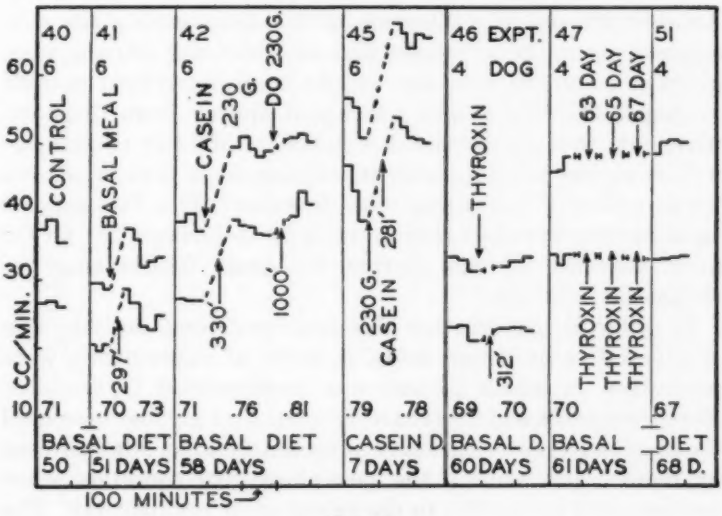

Chart 2

doses of this drug on alternate days, total increases of 45 and 53 per cent, respectively, above the basal clearances of urea and xylose were reached.

It is well known that phlorizin increases the protein metabolism of a fasting dog and we have examined the effects of the drug in the above respect. These experiments are not included in the appended charts. In experiment 61 four control periods yielded the following values on dog 12, which had been on a basal diet for 5 days: (urea clearance/xylose clearance = X) 29.3/41.3 = 0.71. After 3 days of fasting, four

periods yielded average figures of $27.2/36.6 = 0.74$. Two hundred milligrams per kilogram of phlorizin in sodium bicarbonate solution were administered subcutaneously at noon of the day after this experiment and again at midnight. The next morning four periods yielded the average figures $46.5/62.9 = 0.74$. Similar experiments with dog 13 on basal diet for 5 days yielded for four control periods $33.3/42.9 = 0.78$. The dog was fasted the next day and given phlorizin as above. The following day four periods yielded $35.7/50.1 = 0.71$. Another 200 mg. per kilogram of the drug were given subcutaneously in the afternoon and an additional 200 mg. were given at 2 A.M. the next day. Eight hours later, four periods averaged $44.3/62.2 = 0.72$. It would appear from these experiments that an increased metabolism of body protein, as well as an increased metabolism of exogenous protein, acts in the direction of increasing renal function. This fact may be significant in the observations made by Goldring ('31) on the urea clearance of man during the acute febrile stage of rheumatic infection.

In order to test whether this increased renal activity is a specific effect of amino acids, a series of experiments were performed in which glycine was incorporated in the diet. Experiments 78 and 79 (chart 3) show that glycine increased the urea and xylose clearances immediately when administered by mouth, and also in the post-absorptive condition when incorporated in the diet to the extent of 25 gm. per day The effects of subcutaneous and intravenous administration of amino acid were examined in experiments 85 and 86. In these experiments both clearances essentially reached their peak values a few minutes after administration of glycine; the blood urea, however, showed no significant elevation at this time and was still rising at the conclusion of the observations, indicating that the final metabolism of the amino acid is not the effective factor in promoting renal activity. The fact that the clearances rose simultaneously with the blood amino acid, as shown by analyses of the latter that are not included here, indicates that the amino acid may act directly. There

is, however, no proportionality between blood amino acid-nitrogen and increased renal activity.

Experiments 89 and 91 show that glycylglycine and alanine have the same action upon the urea and xylose clearances as glycine, and make it impossible to suppose that the stimulating effect is specific. Glucose, on the other hand (experiment 94), has no effect whatever.

Additional experiments, not to be reported here, have shown that glycolic and pyruvic acids increase both clearances from

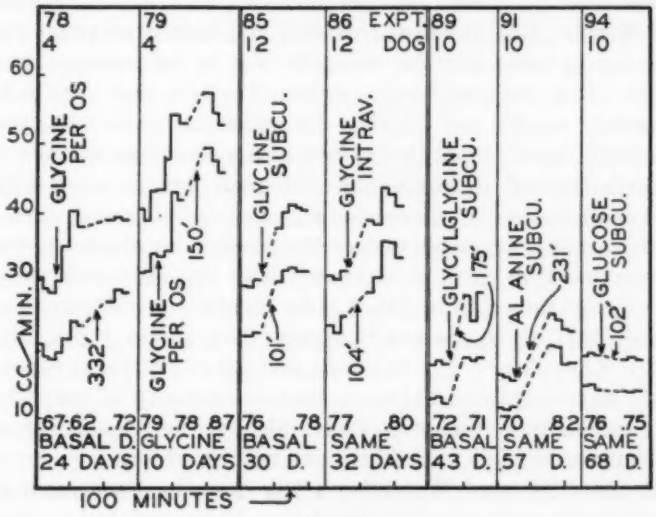

Chart 3

10 to 50 per cent. It seems possible, therefore, that hydroxy acids produced during the metabolism of amino acids may be responsible in part for the increase in renal activity, and consequently it is unwise to attribute prepotency to the amino acid molecule itself.

Other experiments performed in this laboratory have shown that the administration of a salt mixture similar in amount and in composition to the salt content of the meat diet is without effect on the urea and xylose clearances. Large

amounts of sodium sulfate administered intravenously have likewise been shown to exert no appreciable effect on these clearances. It should be reiterated that in all the experiments referred to above, the control observations, as well as the experimental ones, were made with the urine flow above the augmentation limit.

DISCUSSION

If the xylose[1] clearance be accepted as a measure of glomerular filtration, as recommended by Jolliffe, Shannon and Smith ('32), it is evident that the increased renal function can in large part be accounted for by an increase in the latter. It is not possible to explain the facts that the feeding of meat, casein and glycine increase the urea clearances relatively more than the xylose clearance, whereas the administration of thyroxin and phlorizin has no such effect. The explanation of these facts cannot be obtained until an explanation is available of how the glomerular clearance itself is increased, or until it is known why the urea clearance is invariably lower than the xylose clearance. Richards and Plant ('22), Richards and Schmidt ('24), Bieter ('29), White ('30), Khanolkar ('22), Hayman and Starr ('25) and Sheehan ('31) have advanced evidence that the activity of amphibian and mammalian glomeruli is variable, but the reasons for, and mechanism of, this variability are not yet clear.

Brühl ('28) and Watzadse ('28) describe experiments in which failing glomerular circulation in an isolated perfused frog kidney is revived by the addition of glycine. These authors find that amino acids in general, dipeptides, amino acid amides, ereptone, and peptone all increase glomerular activity, and they maintain that the presence of some amino-nitrogen containing substance is necessary for continued

[1] Evidence obtained in this laboratory since this work was completed suggests that there may be a small fraction of the filtered xylose that is actively reabsorbed (compare Shannon, '34). No final opinion on this matter, so far as the dog is concerned, can be given at the present time, but if such proves to be the case the fact of this reabsorption would not modify the significance of the changes in clearance described here.

glomerular circulation. These observations are of particular interest in view of the results which have been reported here.

SUMMARY

The post-absorptive urea and xylose clearances in dogs maintained on a basal diet are low and constant from day to day, and feeding the basal diet has itself little effect on these clearances.

The feeding of meat produces a gradual and prolonged post-prandial rise in both clearances relative to the levels existing before the meal. When meat is used as a maintenance diet over a period of 2 or 3 weeks the post-absorptive clearances are elevated, while the post-prandial effects of eating meat are not obliterated.

A casein diet has qualitatively the same effect in elevating the urea and xylose clearances as has meat. The administration of thyroxin to a dog maintained on a basal diet, and the administration of phlorizin to a fasting dog, likewise elevate the urea and xylose clearances above their respective post-absorptive levels. These facts indicate that protein metabolism as such, whether endogenous or exogenous, is responsible for the increase in renal function.

Feeding a basal diet in which a part of the carbohydrate has been replaced by glycine has the same qualitative effect upon the post-absorptive and post-prandial urea and xylose clearances as has meat; and the subcutaneous or intravenous administration of glycine, glycylglycine and alanine induces immediate but moderate increases in renal activity, whereas glucose is without effect. It would, therefore, appear that the primary effect of protein upon renal function is a result of the action of the amino acids, or of the hydroxy acids produced in the course of their metabolism, upon glomerular activity.

LITERATURE CITED

ADDIS, T., AND D. R. DRURY 1923 The rate of urea excretion. VII. The effect of various other factors than blood urea on the rate of urea excretion. J. Biol. Chem., vol. 55, p. 269.

BIETER, R. N. 1929 The effect of the splanchnics upon glomerular blood flow in the frog's kidney. Am. J. Physiol., vol. 91, p. 436.

BRÜHL, H. 1928 Über die Harnbildung in der Froschniere. XV. Mikroskopische Beobachtungen der Glomerulus funktion in der durchströmten Froschniere. Pflüger's Arch., Bd. 220, S. 380.

COPE, C. L. 1933 Studies of urea excretion. VIII. The effects on the urea clearance of changes in protein and salt contents of the diet. J. Clin. Invest., vol. 12, p. 567.

GOLDRING, W. 1931 Studies of the kidney in acute infection. II. Observations with the urea clearance test in acute rheumatic fever. J. Clin. Invest., vol. 10, p. 345.

GOLDRING, W., L. RAZINSKY, M. GREENBLATT AND S. COHEN 1934 The influence of protein intake on the urea clearance in normal man. J. Clin. Invest., vol. 13, p. 743.

HAYMAN, J. M., JR., AND I. STARR 1925 Experiments on the glomerular distribution of blood in the mammalian kidney. J. Expt. Med., vol. 42, p. 641.

JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH 1932 The excretion of urine in the dog. III. The use of non-metabolized sugars in the measurement of the glomerular filtrate. Am. J. Physiol., vol. 100, p. 301.

KHANOLKAR, V. R. 1922 Partial activity of the kidney and 'the all or nothing' principle. J. Path. and Bact., vol. 25, p. 414.

RICHARDS, A. N., AND O. H. PLANT 1922 Urine formation in perfused kidney. The influence of adrenalin on the volume of the perfused kidney. Am. J. Physiol., vol. 59, p. 184.

RICHARDS, A. N., AND C. F. SCHMIDT 1924 A description of the glomerular circulation in the frog kidney and observations concerning the action of adrenalin and various other substances upon it. Am. J. Physiol., vol. 71, p. 178.

SHANNON, J. A. 1934 The excretion of inulin by the dogfish, Squalus acanthias. J. Cell. and Comp. Physiol., vol. 5, p. 301.

SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH 1932 The excretion of urine in the dog. IV. The effect of maintenance diet, feeding, etc., upon the quantity of glomerular filtrate. Am. J. Physiol., vol. 101, p. 625.

SHEEHAN, H. L. 1931 The deposition of dyes in the mammalian kidney. J. Physiol., vol. 72, p. 201.

VAN SLYKE, D. D. 1927 Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. J. Biol. Chem., vol. 73, p. 695.

WATZADSE, G. 1928 Über die Harnbildung in der Froschniere. XIV. Über die Bedeutung der Aminosäuren für die Nierentätigkeit. Pflüger's Arch., Bd. 219, S. 694.

WHITE, H. L. 1930 Some observations on circulatory changes in a renal glomerulus. Proc. Soc. Expt. Biol. Med., vol. 27, p. 613.

THE VALUE OF THE CHEMICAL TITRATION METHOD IN DETERMINING THE VITAMIN C POTENCY OF CERTAIN FOOD SUBSTANCES[1]

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Recent investigations have shown that vitamin C and ascorbic acid are identical substances (King and Waugh, '32 a and b; Harris and Ray, '33; Herbert, Hirst, Percival, Reynolds and Smith, '33; Reichstein, Grussner and Oppenauer, '33; Ault, Baird, Charrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey, '33) and that ascorbic acid can be estimated quantitatively by titrating with a standard solution of iodine or a standard solution of 2, 6-dichlorophenol-indophenol.

A review of the literature relative to the chemical estimation of vitamin C in foodstuffs (Tillmans and Hirsch, '32; Wolff, Kekeln and Emmerie, '33; Birch, Harris and Ray, '33; Bessey and King, '33) indicated that the method proposed by Bessey and King is the least affected by interfering substances; consequently, this method was used in the following studies.

The present investigation was conducted with the view of attempting to establish further correlations between chemical titration methods and the usual biological method for the estimation of vitamin C in fruit and vegetable juices.

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, December 6, 1934, as technical paper no. 669.

EXPERIMENTAL

The studies herein reported consist of the determination of the ascorbic acid content of several fruit and vegetable juices by the chemical method and the subsequent comparison of some of the results obtained by the conventional biological method of vitamin C assay.

Chemical assay

Bessey and King ('33) have pointed out that lemon juice could be used with reasonable accuracy in the standardization of the dye, while Birch, Harris and Ray ('33) stated that both lemon and orange juice are reliable in this respect.

In these studies, lemon juice was always used in the standardization of the dye solution. The technic used is as follows: The dye solutions were prepared just previous to being used by dissolving 0.1 gm. of the dye in successive portions of warm water, diluting to 200 ml., then cooling and filtering. A 5 ml. aliquot of the freshly prepared and strained lemon juice was titrated with 0.01 normal iodine solution until a permanent bluing of the starch indicator resulted. Ten milliliters of an 8 per cent acetic acid solution were added to a second 5 ml. aliquot of the lemon juice and the dye solution run in from a burette until the end point (pink) was reached. By means of this technic, interfering substances which fade the dye slowly are easily detected when the end point has been reached.

Since 1 ml. of 0.01 normal iodine solution reacts with 0.88 mg. of ascorbic acid, the ascorbic acid equivalent of the dye solution was easily calculated. Other juices were then evaluated as to their ascorbic acid content by titrating against this dye solution of known titer.

For the purpose of reference, the dye solutions used were standardized occasionally against pure ascorbic acid as well as against several different fruit juices. In table 1 are given the results obtained by standardizing some of the dye solution against ascorbic acid, lemon juice, orange juice, and grapefruit juice. The results are expressed as milligrams of ascorbic acid for each milliliter of the dye solution.

The above results indicated that lemon juice and grapefruit juice could be used as standards without introducing serious errors in the technic. Orange juice, however, appeared to be less reliable in this respect. While the juice of only one orange was titrated in direct comparison with a known amount of ascorbic acid on each of the above days, other titrations of orange juice showed about the same irregularities when lemon juice was used as the check standard.

Biological assay

The biological assays were carried out according to the technic of Sherman, LaMer and Campbell ('22). Young guinea pigs weighing from 250 to 300 gm. were used as the

TABLE 1

Showing the results obtained by standardizing some of the dye solutions against ascorbic acid, lemon juice, orange juice and grapefruit juice

DATE	ASCORBIC ACID STANDARDIZATION	LEMON JUICE STANDARDIZATION	ORANGE JUICE STANDARDIZATION	GRAPEFRUIT JUICE STANDARDIZATION
4/25/34	0.147 [1]	0.155	0.165	....
5/24/34	0.149 [2]	0.150	0.160	0.150
6/26/34	0.144 [3]	0.152	....	0.146

[1] Natural ascorbic acid furnished through the courtesy of Dr. C. G. King.
[2] Natural ascorbic acid furnished through the courtesy of Merck & Co.
[3] Synthetic ascorbic acid furnished through the courtesy of Hoffmann-LaRoche & Co.

experimental animals. These pigs were kept in individual metal cages which were provided with raised screen floors. With the exception of a slight modification of the scorbutic diet, these animals were fed and handled according to the technic of the above authors. The basal scorbutic diet used in these studies contained 2 per cent of cod liver oil (Patch's medicinal grade) and 8 per cent of butter fat instead of the usual 10 per cent of butter fat. This alteration in the composition of the scorbutic diet was made in order to insure a liberal supply of vitamin D.

The first series of biological assays for vitamin C were made on fresh Florida orange juice and canned pineapple juice. A week's supply of the fresh fruit and the canned juice

was kept in the refrigerator at all times during the test period. The juice of the oranges was extracted just previous to titration and feeding. The canned pineapple juice was opened just before being used.

Shrader and Johnson ('34) and Nelson and Molten ('34) had shown that when fruit juices are kept frozen for long periods of time, there is no appreciable loss of antiscorbutic potency. Consequently, for the second series of biological assays, composite samples of the various juices were preserved in the above manner until the assays could be made. In the case of lemon juice, 5 dozen of each of three commercial grades of lemons were used as the source of juice. One dozen select grapefruits and 1 dozen fresh pineapples were used as sources of these juices. The fruits were pared by hand and the juices pressed out mechanically. The juices were strained and placed in small waxed-cardboard containers, each of which held an ample supply for daily experimentation, and then stored at a temperature of —10°F. The freshly thawed juices were fed to guinea pigs on the basis of the calculated minimum protective dosage as determined by the dye titer. It was hoped that by such a procedure, a uniform supply of the various juices could be had for the entire experimental period, and that results could be obtained which would show whether or not the calculated levels of these juices furnished the desired degree of protection to guinea pigs, and, further, that some information might be obtained concerning a possible variation in the antiscorbutic potency of juices from different grades of lemons. The stored juices were titrated from time to time during the storage period against a standard dye solution. The resultant titers showed that no marked decrease in vitamin C potency took place during the 90-day storage period.

Bessey and King ('33) have shown that 0.5 mg. of ascorbic acid daily per guinea pig prevented scurvy, allowed satisfactory survival, and maintained the experimental animals in a condition of reasonable sensitivity to changes in vitamin intake. Therefore, in these studies the calculations have been based on the assumption that 0.5 mg. of ascorbic acid or its

equivalent is the minimum protective dose for the average size experimental guinea pig.

DATA AND DISCUSSION

A condensed summary of the data obtained in these studies is given in the following tables.

Table 2 gives the results obtained in a series of biological assays that were made on canned pineapple juice and fresh

TABLE 2

Showing the results obtained by feeding varying amounts of canned pineapple juice and fresh orange juice to groups of young guinea pigs that were receiving a scurvy-producing diet

ANIMAL GROUP NO.	NUMBER OF ANIMALS CONSIDERED	SOURCE OF VITAMIN C (JUICES)	QUANTITY OF JUICE FED DAILY	TITRATION EQUIVALENT IN MILLIGRAMS OF ASCORBIC ACID	THEORETICAL[1] INTERNATIONAL UNITS	AVERAGE DAILY GAIN IN WEIGHT	SCURVY SCORE
			ml.			*gm.*	
1	6	Pineapple	0	0	0	All died	Severe
2	6	Pineapple	3.0	0.245	5.7	All died	Severe
3	3	Pineapple	4.0	0.352	7.6	0	17
4	4	Pineapple	5.0	0.440	9.5	0.5	8
5	9	Pineapple	6.0	0.528	11.4	1.8	3
6	7	Pineapple	7.0	0.616	13.3	2.4	0–2
7	5	Pineapple	8.0	0.707	15.2	2.7	0
8	8	Pineapple	9.0	0.792	17.1	2.7	0
9	7	Pineapple	10.0	0.880	19.0	2.9	0
10	4	Orange	1.0	0.500	11.0	2.8	0–2
11	9	Orange	1.5	0.750	16.5	3.0	0
12	9	Orange	2.0	1.000	22.0	3.2	0
13	8	Orange	3.0	1.500	33.0	3.2	0
14	6	Orange	4.0	2.000	44.0	3.6	0
15	5	Orange	5.0	2.500	55.0	3.7	0

[1] Calculations based on the average value (1.1 ml. = 0.5 mg. of ascorbic acid) obtained by titrating ten different samples of lemon juice.

orange juice. The table also contains the equivalent ascorbic acid content of the respective quantities of the two juices fed, as determined by the dye titration method. In addition, the theoretical International units of vitamin C are given.

The six animals comprising group 1, which served as a negative control, were all dead by the thirty-first day, while those animals comprising group 2 were all dead by the forty-second day. On autopsy, all animals of these two groups were

found to exhibit symptoms of severe scurvy. Although the animals of groups 3 and 4 survived the experimental period, they likewise showed symptoms of severe scurvy. The animals of group 5 were somewhat irregular in their responses and, when autopsied, their scurvy score ranged from 1 to 6, with an average score of 3.

The animals of group 6 showed only very mild symptoms of scurvy and their growth rates were not significantly depressed. Due to these facts, this group of animals was considered as having received a minimum protective dose of vitamin C or the equivalent of 0.5 mg. of ascorbic acid daily.

The animals of groups 7, 8 and 9 all showed practically the same growth rate and survived the experimental period without showing any evidence of scurvy.

The animals of group 10, which received orange juice, made a similar response to that of those animals comprising group 6, which received the canned pineapple juice. From this it appeared that 1 ml. of fresh orange juice was equivalent to about 7 ml. of canned pineapple juice in antiscorbutic potency. Results obtained by the dye titration had indicated that the relative antiscorbutic potencies of the two juices would be about 6 : 1.

The animals of groups 11, 12, 13, 14 and 15 showed no evidence of scurvy during the experimental period and when autopsied they appeared to be normal in every respect.

In table 3 are given the results of a second series of biological assays. It may be observed from the data given in this table that the biological responses of the several groups of animals were quite uniform. In every case, with the exception of one group, the calculated dosage gave the expected response. The animals of group 16, which received the calculated sub-protective dosage of fresh pineapple juice, did not show as severe scurvy symptoms as might have been expected from the results given in a previous publication on this subject (Bessey and King, '33).

Table 4 contains the data obtained by titrating the juices of several fruits and vegetables against a standard solution

TABLE 3

Showing the biological responses made by young guinea pigs receiving a scorbutic diet when this diet was supplemented by such quantities of the various fruit juices as would furnish a definite amount of ascorbic acid

ANIMAL GROUP NO.	NUMBER OF ANIMALS CONSIDERED	SOURCE OF VITAMIN C (JUICES)	QUANTITY OF JUICE FED DAILY	TITRATION EQUIVALENT IN MILLIGRAMS OF ASCORBIC ACID	INTERNATIONAL UNITS	AVERAGE DAILY GAIN IN WEIGHT	SCURVY SCORE
			ml.			*gm.*	
16	3	Pineapple	1.0	0.3	6.6	2.4	6
17	5	Pineapple	1.6	0.5	11.0	2.4	0–2
18	5	Pineapple	2.2	0.7	15.4	3.4	0
19	4	Lemon I	1.1	0.5	11.0	2.8	0–2
20	3	Lemon II	1.1	0.5	11.0	2.5	0–2
21	5	Lemon III	1.0	0.5	10.0	2.8	0–2
22	6	Grapefruit	1.9	0.5	11.0	2.7	0–2

TABLE 4

Showing the ascorbic acid equivalent and the calculated minimum protective dose of various fruit and vegetable juices as determined by titrating against a standardized solution of 2, 6-dichlorophenolindophenol

KINDS OF JUICE USED	QUALITY OF JUICE	NUMBER OF SAMPLES CONSIDERED	ASCORBIC ACID EQUIVALENT	CALCULATED MINIMUM PROTECTIVE DOSAGE (AVERAGE)
			(*mg. per ml.*)	(*ml.*)
Lemon	Fresh	13	0.455	1.1
Orange	Fresh	10	0.500	1.0
Orange	Canned	2	0.294	1.7
Tangerine	Fresh	2	0.208	2.4
Lime	Fresh	1	0.459	1.1
Grapefruit	Fresh	1	0.266	1.9
Grapefruit	Canned	1	0.410	1.2
Pineapple	Fresh	2	0.310	1.6
Pineapple	Canned	18	0.070	7.2
Tomato	Fresh	1	0.157	3.2
Tomato	Canned	1	0.242	2.1
Peach	Fresh	1	0.018	27.8
Plum	Fresh	1	0.030	16.7
Rhubarb	Fresh	1	0.278	1.8
Sauerkraut	Canned	1	0.082	6.2
Cabbage	Fresh	1	0.427	1.2
Peppers	Green	1	0.203	2.5
Carrots	Fresh	1	0.040	12.5
Celery	Fresh	1	0.009	55.6
Turnip	Fresh	1	0.191	2.6
Cucumber	Fresh	1	0.012	41.6
Potato	Fresh	1	0.021	23.8

of the dye. In this table, the results are expressed in terms of the ascorbic acid equivalent in milligrams per milliliter of juice and as the quantity required to furnish a minimum protective dose.

SUMMARY

A study has been made concerning the value of the chemical titration method for determining the vitamin C potency of certain food substances. In these studies, the ascorbic acid content of certain fruit and vegetable juices was determined by titrating against a standard solution of 2, 6-dichlorophenol-indophenol. In order to ascertain the accuracy of the data obtained through the chemical titration method, biological assays were also made on a number of the fruit juices used in this study. From the results obtained it appears that:

1. The chemical titration method for the estimation of ascorbic acid is of material value in determining the vitamin C potency of certain fruit and vegetable juices. By means of this method, it is possible at least to approximate the antiscorbutic activity of the juices before the biological assay is started, thus saving time and expense.

2. The accuracy of the results obtained by the chemical titration method appears to depend to an appreciable degree upon the nature of the juice that is being titrated. Some juices contain interfering substances that react with the dye, thus complicating the titration results and leading to erroneous conclusions.

3. For lemon juice, orange juice, grapefruit juice and fresh pineapple juice, the values obtained by the chemical titration method are in close agreement with those obtained by the usual biological assay method.

4. Fresh pineapple juice, when evaluated for its antiscorbutic potency by the chemical method, gives values in closer agreement with those obtained by the biological method than does the canned pineapple juice, owing to the presence of interfering substances in the latter.

LITERATURE CITED

AULT, G. R., D. K. BAIRD, H. C. CHARRINGTON, W. N. HAWORTH, R. HERBERT, E. L. HIRST, E. G. V. PERCIVAL, F. SMITH AND M. STACEY 1933 Synthesis of d- and l-ascorbic acid and analogous substances. J. Chem. Soc., vol. 2, p. 1419.

BESSEY, O. A., AND C. G. KING 1933 The distribution of vitamin C in plant and animal tissues and its determination. J. Biol. Chem., vol. 103, p. 687.

BIRCH, T. W., J. L. HARRIS AND S. N. RAY 1933 A microchemical method for determining the hexuronic acid (vitamin C) content of foodstuffs. Biochem. J., vol. 27, p. 595.

HARRIS, J. L., AND S. N. RAY 1933 Specificity of hexuronic acid as an antiscorbutic factor. Biochem. J., vol. 27, p. 580.

HERBERT, R. W., E. L. HIRST, E. G. V. PERCIVAL, R. J. REYNOLDS AND F. SMITH 1933 The constitution of ascorbic acid. J. Chem. Soc., vol. 2, p. 1270.

KING, C. G., AND W. A. WAUGH 1932 a Chemical nature of vitamin C. Science, vol. 75, p. 357.

———— 1932 b Isolation and identification of vitamin C. J. Biol. Chem., vol. 97, p. 325.

NELSON, E. M., AND H. M. MOLTEN 1934 Studies of vitamin C in citrous fruits. Abstracts of the divisions of Agricultural and Food Chemistry, American Chemical Society, St. Petersburg, Florida.

REICHSTEIN, T., A. GRUSSNER AND R. OPPENAUER 1933 Synthese der d- und l-ascorbinsäure. Helv. Chim. Acta., Bd. 16, S. 1019.

SHERMAN, H. C., V. K. LAMER AND H. L. CAMPBELL 1922 The quantitative determination of the antiscorbutic vitamin. J. Am. Chem. Soc., vol. 44, p. 165.

SHRADER, J. H., AND H. H. JOHNSON 1934 Preservation of orange juice by freezing. Abstracts of the Divisions of Agricultural and Food Chemistry, American Chemical Society, St. Petersburg, Florida.

TILLMANNS, J., AND P. HIRSCH 1932 Über das vitamin C. Biochem. Z., Bd. 250, S. 312.

WOLFF, L. K., M. v. KEKELN AND A. EMMERIE 1933 Demonstration of the quantitative estimation of vitamin C. Acta Brevia Neerland. Physiol., Pharmacol., Microbiol., vol. 3, p. 44. C. A., vol. 27, p. 3964.

UTILIZATION OF MEAT BY HUMAN SUBJECTS [1]

II. THE UTILIZATION OF THE NITROGEN AND PHOSPHORUS OF ROUND AND LIVER OF BEEF

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Mitchell ('27) has suggested that the nitrogen of beef liver has a higher biological value for rats than that of beef round, attributing the difference to some extent to relatively larger amounts of connective tissue in the round. The study here reported was made to see if similar results for nitrogen would be obtained with human subjects and to determine the utilization of the phosphorus from the two meats. Samples were also analyzed for collagen and elastin nitrogen, so the relationship of these factors to the utilization of total nitrogen and phosphorus might be studied.

The subjects were three healthy young college women, ranging in weight from 56 to 60 kg., with activities varying from light to moderate exercise. Subjects A and B consumed 39 and subject C 42 Calories per kilogram daily, amounts sufficient to maintain their weights.

The experiment was divided into two parts, one for each meat. Each part consisted of a 4-day preliminary period followed by two 4-day experimental periods. The meats[2] were of good grade and of known history. Equal weights of liver and round were used from the same animal, thus eliminating differences due to grade. Each meat was freed from visible fat, coarsely ground once to permit of sampling, and

[1] Contribution no. 37, Department of Home Economics.

[2] The authors wish to thank Prof. David Mackintosh, of the Department of Animal Husbandry of Kansas State College, for supplying the meats.

mixed well. Weighed portions for every lunch and dinner for each individual were wrapped separately and kept frozen until used. Then they were browned without fat and cooked slowly in individual pans in a small amount of distilled water.

The diet (table 1) consisted of the meat to be tested, a low-nitrogen bread of cracker-like texture, filtered butter fat,

TABLE 1

Daily food intake

SUBJECT	FOOD	BEEF ROUND PERIOD				BEEF LIVER PERIOD			
		Weight	Protein	Phosphorus	Calories	Weight	Protein	Phosphorus	Calories
		gm.	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	
A and B	Meat	131.0	28.81	0.232	204	136.8	30.88	0.493	176
	Bread [1]		0.53		927		0.53		927
(56 kg.	Butterfat,								
each)	filtered	17.0			153	20.5			185
	Beverage [2]	1120.0	11.75	0.188	910	1120.0	8.75	0.179	910
	Calcium								
	carbonate	0.550				0.538			
	Ferric								
	citrate	0.043				0.008			
	Sodium								
	chloride	3.000				3.000			
	Total		41.09	0.420	2194		40.16	0.672	2198
C	Meat	143.2	31.63	0.253	223	149.5	33.69	0.539	193
	Bread [1]		0.53		927		0.53		927
(60 kg.)	Butterfat,								
	filtered	15.0			135	18.5			167
	Beverage [2]	1120.0	11.75	0.188	910	1120.0	8.75	0.179	910
	Candy [3]				321				321
	Calcium								
	carbonate	0.680				0.643			
	Ferric								
	citrate	0.042				0.004			
	Sodium								
	chloride	3.000				3.000			
	Total		43.91	0.441	2516		42.97	0.718	2518

[1] Bread: cornstarch, 120 gm.; lactose, 40 gm.; Dextri-maltose, 15 gm.; baking powder, tartrate, 3 gm.; sodium chloride, 3 gm.; Agar, 4 gm.; butterfat, filtered, 32 gm.; water, distilled, 80 gm.

[2] Beverage: orange juice, 1000 gm.; lactose, 80 gm.; sucrose, 40 gm.

[3] Candy: sucrose, 70 gm.; cornstarch, 4 gm.; butterfat, filtered, 3 gm.; water, distilled, 60 gm.

and orange juice with lactose and sucrose. Subject C also ate a fondant-like candy. During the liver period the butter fat was increased slightly to maintain the caloric intake; otherwise the two diets were the same, except for the meat. Both diets were base-forming. Distilled water was used in approximately constant amounts by each subject, ranging from 650 to 1000 cc. daily, according to individual preferences. The sodium chloride content of the diet was also controlled. Calcium and iron were brought up to accepted standards (Sherman, '32) by addition of calcium carbonate and ferric citrate.

Analysis of the foods used showed the meats to be somewhat higher in nitrogen and the orange juice and cornstarch much higher than expected. This placed the nitrogen somewhat above the calculated level of 85 per cent of the protein requirement suggested by Sherman ('32), i.e., 44.4 gm. per 70 kg. of body weight, and reduced the percentage supplied by the meats to an average of approximately 71 per cent of the total nitrogen for the beef round period and to 77 per cent for the beef liver. However, since the protein level was still low and the proportion supplied by the meat high, it is believed the results obtained may be attributed largely to the meat consumed.

The round supplied an average of 56 per cent of the total dietary phosphorus and the liver 74 per cent—amounts lower than desired, but still providing the major portion of the phosphorus in the diet. On the basis of 0.88 gm. phosphorus daily per 70 kg. of body weight (Sherman, '32), the phosphorus intake in the beef round period averaged only 59 per cent of the amount needed by the different subjects, whereas when liver was eaten, the requirement was somewhat more than met.

Urine was collected quantitatively for each 24-hour day and feces for each 4-day period. The usual precautions were used in caring for samples. Total nitrogen was determined by the Kjeldahl-Gunning procedure; phosphorus by the volumetric method of the Association of Official Agricultural Chemists;

and collagen and elastin nitrogen by a revised method from the Illinois Agricultural Experiment Station.

All subjects were in positive nitrogen balance with both meats (table 2). With an average daily intake of approximately 0.12 and 0.11 gm. nitrogen per kilogram, respectively, for the two meats, the average daily retention for the 8 experimental days of the beef round period was $+ 0.91 \pm 0.11$ and for the beef liver $+ 0.82 \pm 0.11$ gm. per person or $+ 0.016$ and $+ 0.014$ gm. per kilogram. For the 24 experimental days on each meat for the entire group, there were twenty-two positive balances for round and twenty-one for liver. Two subjects had negative balances in each case.

The average coefficients of digestibility for the nitrogen for the various subjects on beef round ranged from 79.3 to 84.8 per cent and averaged 82.5 per cent for all subjects. With beef liver it ranged from 73.7 to 86.6 per cent and averaged 82.1 per cent, which was practically the same as for round. Subjects B and C had higher coefficients of digestibility for liver nitrogen, but subject A digested round better. The lowest values for both meats were for subject A indicating that individual differences are an important factor. Although the diet contained more nitrogen when round was eaten, no difference in urinary nitrogen output was observed. It averaged 0.08 gm. per kilogram of body weight per day for each kind of meat; again suggesting that the nitrogen of the two meats was used about equally well. Any differences observed apparently were not significant.

Both collagen and elastin nitrogen were lower in liver than in round of beef (table 2). Since any difference in utilization of these two meats was slightly in favor of beef round, something other than connective tissue must have influenced the results.

The average phosphorus balance for all subjects for both meats was slightly negative amounting to -0.035 ± 0.015 gm. per person per day for the round and -0.010 gm. ± 0.020 gm. for the liver which, in the latter case, was approximate equilibrium. Two subjects were in negative phosphorus

TABLE 2

Average daily nitrogen and phosphorus balances

SUBJECT	CUT OF MEAT	AVERAGE DAILY INTAKE		AVERAGE DAILY OUTPUT			BALANCE		
		Meat	Total	Urine	Feces	Total	Total	Per kg.	Per 70 kg.
Nitrogen [1]									
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A	Round [2]	4.61	6.57	4.11	1.36	5.47	+ 1.10 ± 0.13	+ 0.020	+ 1.40
(56 kg.)	Liver [3]	4.94	6.42	4.27	1.69	5.96	+ 0.46 ± 0.26	+ 0.008	+ 0.56
B	Round	4.61	6.57	4.84	1.10	5.94	+ 0.63 ± 0.22	+ 0.011	+ 0.77
(56 kg.)	Liver	4.94	6.42	4.48	0.89	5.37	+ 1.05 ± 0.17	+ 0.019	+ 1.33
C	Round	5.06	7.03	4.94	1.07	6.01	+ 1.02 ± 0.25	+ 0.017	+ 1.19
(60 kg.)	Liver	5.39	6.88	5.01	0.92	5.93	+ 0.95 ± 0.12	+ 0.016	+ 1.12
Average for	Round	4.76	6.72	4.63	1.18	5.81	+ 0.91 ± 0.11	+ 0.016	+ 1.12
all subjects	Liver	5.09	6.57	4.59	1.17	5.75	+ 0.82 ± 0.11	+ 0.014	+ 0.98
(57.3 kg.)									
Phosphorus [1]									
								mg.	
A	Round	0.232	0.420	0.242	0.175	0.417	+ 0.003 ± 0.007	+ 0.05	+ 0.004
(56 kg.)	Liver	0.493	0.672	0.499	0.224	0.723	− 0.051 ± 0.060	− 0.91	− 0.064
B	Round	0.232	0.420	0.277	0.199	0.476	− 0.056 ± 0.012	− 1.00	− 0.070
(56 kg.)	Liver	0.493	0.672	0.441	0.219	0.660	+ 0.012 ± 0.027	+ 0.21	+ 0.015
C	Round	0.253	0.441	0.262	0.230	0.492	− 0.051 ± 0.046	− 0.85	− 0.060
(60 kg.)	Liver	0.539	0.717	0.438	0.269	0.707	+ 0.010 ± 0.024	+ 0.17	+ 0.012
Average for	Round	0.239	0.427	0.260	0.201	0.462	− 0.035 ± 0.015	− 0.60	− 0.042
all subjects	Liver	0.508	0.687	0.459	0.237	0.697	− 0.010 ± 0.020	− 0.18	− 0.013
(57.3 kg.)									

[1] Average of 8 experimental days.

[2] Collagen furnished 6.69 per cent of total nitrogen; elastin 0.29 per cent.

[3] Collagen furnished 3.07 per cent of total nitrogen; elastin 0.20 per cent.

(Analyses for collagen and elastin were made by the Chemistry Laboratory of the Kansas Agricultural Experiment Station.)

balance on round, whereas only one was negative on liver, but when round was eaten the diet averaged 7.4 mg. phosphorus per kilogram of body weight; in the liver period, 12.0 mg. per kilogram. Both fecal and urinary phosphorus were higher for all subjects when liver was eaten, as was to be expected with the higher intake in this period. However, fecal phosphorus was lowered from an average of 43.5 per cent for round to 34.0 per cent for liver, which suggests superior absorption of liver phosphorus.

The large amounts of orange juice and lactose in the diet probably favored the use of the phosphorus, since they are believed to increase acidity in the digestive tract and raise absorption accordingly (Bergeim, '26; Chaney and Blunt, '25). The basic reaction of the foods used may also have had an effect. On the other hand, the diet was low in vitamin D and the ultraviolet light received by the subjects was limited—factors unfavorable to best usage of phosphorus—as was a probable disproportion in the ratio of phosphorus to calcium.

SUMMARY

Three young women apparently used the nitrogen of round of beef fully as well as that from liver of beef, as indicated by almost identical coefficients of digestibility and a slightly more favorable nitrogen balance during the period round was eaten. The latter may be accounted for by a slightly higher intake.

The phosphorus balance was but little lower on round, although the diet during this period supplied less than two-thirds as much phosphorus. A higher percentage of the liver phosphorus may have been absorbed.

The collagen and elastin were higher in round than in liver, so apparently could not have affected seriously the utilization of the total nitrogen of this cut.

LITERATURE CITED

BERGEIM, O. 1926 Intestinal chemistry. V. Carbohydrates and calcium and phosphorus absorption. J. Biol. Chem., vol. 70, p. 35.

CHANEY, M., AND K. BLUNT 1925 The effect of orange juice on the calcium, phosphorus, magnesium, and nitrogen retention and urinary organic acids of growing children. J. Biol. Chem., vol. 66, p. 829.

MITCHELL, H. H. 1927 The protein values of foods in nutrition. J. Home Econ., vol. 19, p. 122.

SHERMAN, H. C. 1932 Chemistry of food and nutrition, 4th ed. The Macmillan Company, New York.

UTILIZATION OF MEAT BY HUMAN SUBJECTS [1]

III. THE UTILIZATION OF THE NITROGEN AND PHOSPHORUS OF BEEF HEART

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Beef heart has been reported (Mitchell, '27) to have a biological value for rats somewhat lower than liver, but superior to round. To investigate the value of beef heart for human subjects, the utilization of its nitrogen and phosphorus has been studied by means of balance experiments. Beef round was used as a control. The subjects, four healthy young college women, maintained their weights (table 2) throughout the experiment on an intake of 45 Calories per kilogram.

The diet (table 1) and procedure for conducting the experiment were essentially the same as in earlier studies (Long and Pittman, '35). A good grade of beef of known origin was used. The heart and an equal weight of round were taken from each of eleven carcasses. The meats[2] were prepared, stored, and cooked as previously described.

The diet furnished approximately the daily protein requirement of 44.4 gm. per 70 kg. (Sherman, '32) for the beef round period and from 13 to 18 per cent in excess of this during the beef heart period. The daily phosphorus intake averaged 67 per cent of the requirement of 0.88 gm. per 70 kg. (Sherman, '32) for the beef round period and 88 per cent for the

[1] Contribution no. 42, Department of Home Economics.

[2] The authors wish to thank Prof. David Mackintosh, of the Department of Animal Husbandry of Kansas State College, for supplying the meats.

TABLE 1

Daily food intake

SUBJECT	FOOD	BEEF ROUND PERIOD				BEEF HEART PERIOD			
		Weight	Protein	Phosphorus	Calories	Weight	Protein	Phosphorus	Calories
		gm.	gm.	gm.		gm.	gm.	gm.	
A	Meat	113.6	21.9	0.203	132	151.4	24.9	0.319	176
	Bread [1]		0.4		927		0.4		927
	Butterfat, filtered	20.0			180	15.0			135
	Beverage [2]	1120.0	9.5	0.205	910	1120.0	10.0	0.216	910
	Calcium carbonate	0.400				0.399			
	Ferric citrate	0.024				0.020			
	Sodium chloride	4.000				4.000			
	Total		31.8	0.408	2149		35.3	0.535	2148
B	Meat	125.6	24.1	0.225	146	167.3	27.5	0.353	194
	Bread [1]		0.4		927		0.4		927
	Butterfat, filtered	43.2			389	38.0			342
	Beverage [2]	1120.0	9.5	0.206	910	1120.0	10.0	0.217	910
	Calcium carbonate	0.518				0.517			
	Ferric citrate	0.028				0.023			
	Sodium chloride	4.000				4.000			
	Total		34.0	0.431	2372		37.9	0.570	2373
C	Meat	127.8	24.4	0.229	148	170.0	28.1	0.359	197
	Bread		0.4		927		0.4		927
	Butterfat, filtered	47.7			429	42.0			378
	Beverage	1120.0	9.5	0.205	910	1120.0	10.0	0.215	910
	Calcium carbonate	0.539				0.538			
	Ferric citrate	0.028				0.024			
	Sodium chloride	4.000				4.000			
	Total		34.3	0.434	2414		38.5	0.574	2412
D	Meat	131.5	25.0	0.236	153	175.2	28.8	0.369	203
	Bread		0.4		927		0.4		927
	Butterfat, filtered	55.0			495	49.3			444
	Beverage	1120.0	9.5	0.206	910	1120.0	10.0	0.217	910
	Calcium carbonate	0.577				0.585			
	Ferric citrate	0.029				0.025			
	Sodium chloride	4.000				4.000			
	Psyllium seed	18.000	2.1	0.068		18.000	2.2	0.068	
	Total		37.0	0.510	2485		41.4	0.654	2484

[1] Bread: cornstarch, 120 gm.; lactose, 40 gm.; dextri-maltose, 15 gm.; baking powder, tartrate, 3 gm.; sodium chloride, 3 gm.; agar, 5 gm.; butterfat, filtered, 32 gm.; water, distilled, 80 gm.

[2] Beverage: orange juice, 1000 gm.; lactose, 80 gm.; sucrose, 40 gm.

beef heart period. The unexpectedly high nitrogen content of the orange juice and the difference in the amount of nitrogen in the meats from the calculated values made the nitrogen level of the diet higher than was desired and reduced the proportion supplied by the meats. The meats actually furnished an average of only 70 per cent of the total nitrogen and 50 per cent of the total phosphorus for all subjects in the beef round period and 71 per cent of the total nitrogen and 60 per cent of the total phosphorus when beef heart was eaten.

The methods of collection, preservation, and analysis were the same as described by Pittman ('32).

The average nitrogen balance for all subjects was slightly positive for both meats, amounting to $+ 0.02 \pm 0.12$ gm. for round and $+ 0.60 \pm 0.10$ gm. for heart. The difference between the values for the two meats is of doubtful significance ($\frac{0.58}{0.16} = 3.6$) and may be due to the level at which the meats were fed rather than to quality of nitrogen. Three subjects showed a negative balance for the 8 days of the round period on an intake of 0.11 gm. nitrogen per kilogram, whereas all were in positive balance when heart was eaten at the somewhat higher level of 0.12 gm. per kilogram. There were fifteen positive balances for round and twenty-seven for heart out of 32 and 31 experimental days, respectively. Long and Pittman ('35) obtained, with beef round on a similar diet, an average balance of $+ 0.91 \pm 0.11$ gm. with an intake of 0.12 gm. nitrogen per kilogram. The average coefficients of digestibility for nitrogen in the present study were practically identical for the two meats approximating 87 per cent for both. As noted in other experiments, variations in the same subject from day to day and individual differences were greater than those noted between meats.

Urinary nitrogen averaged 0.089 gm. per kilogram daily for all subjects with each cut of meat and, as coefficients of digestibility were also similar, it appears that retention of nitrogen from the two meats was equally good, though the round supplied a smaller amount.

The heart contained more than twice as much collagen[3] as the round and but little more elastin (table 2). As heart showed somewhat higher balances for both nitrogen and phosphorus than round and was equally well digested, its larger amount of connective tissue apparently did not decrease its value to a noticeable degree. This beef round was approximately 25 per cent higher in both forms of connective tissue than that used by Long and Pittman ('35) which may help to explain the higher balance they obtained.

The average daily phosphorus balance for all subjects on beef round with an intake averaging 8.5 mg. per kilogram was -0.054 ± 0.009 gm. During the heart period, with intake averaging 11.1 mg. per kilogram, the average daily balance was more favorable, amounting to $+0.002 \pm 0.010$ gm. While the difference between the two values may appear to be significant ($\frac{0.056}{0.009} = 6$), it is probable that it was also due to the level at which the meats were fed. All subjects were in negative phosphorus balance on beef round; with heart, as was to be expected with a higher intake, all approached equilibrium, two being slightly negative and two slightly positive. Superior quality of meat phosphorus is suggested when it is compared with that from navy beans (Pittman, '32) where, with an intake slightly higher per unit of weight than the highest for meat, the balance was considerably more negative. The results reported by Long and Pittman ('35) showed a slightly more favorable usage of beef round phosphorus on a somewhat lower intake per kilogram than was obtained in these experiments. Their values for beef liver, on a somewhat higher intake of phosphorus per unit of weight, were close to these for beef heart.

With one exception, both urinary and fecal phosphorus were increased in the beef heart period, though the percentage distribution did not change greatly. It appears that absorption of phosphorus from the two meats was not widely different.

[3] Analyses were made by the Chemistry Laboratories of the Kansas Agricultural Experiment Station.

TABLE 2

Average daily nitrogen and phosphorus balances

SUBJECT	CUT OF MEAT	AVERAGE DAILY INTAKE		AVERAGE DAILY OUTPUT			BALANCE		
		Meat	Total	Urine	Feces	Total	Total	Per kg.	Per 70 kg.
Nitrogen [1]									
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
A	Round [2]	3.49	5.06	4.64	0.64	5.28	−0.22 ± 0.26	−0.005	−0.35
(47 kg.)	Heart [2]	3.98	5.65	4.16	0.93	5.09	+0.56 ± 0.11	+0.012	+0.84
B	Round	3.86	5.43	5.03	0.74	5.77	−0.34 ± 0.33	−0.006	−0.42
(52.7 kg.)	Heart	4.40	6.05	4.58	0.60	5.18	+0.87 ± 0.28	+0.016	+1.12
C	Round	3.92	5.50	4.94	0.61	5.55	−0.05 ± 0.07	−0.001	−0.07
(53.6 kg.)	Heart	4.47	6.14	4.98	0.83	5.81	+0.33 ± 0.11	+0.006	+0.42
D [4]	Round	4.04	5.96	4.13	1.14	5.27	+0.69 ± 0.18	+0.012	+0.84
(55.2 kg.)	Heart	4.61	6.63	4.91	1.03	5.94	+0.69 ± 0.08	+0.012	+0.84
All subjects	Round	3.83	5.49	4.63	0.78	5.41	+0.02 ± 0.12	±0.000	±0.00
(52.3 kg.)	Heart	4.36	6.12	4.65	0.86	5.52	+0.60 ± 0.10	+0.011	+0.77
Phosphorus [1]									
								mg.	
A	Round	0.203	0.408	0.315	0.168	0.483	−0.075 ± 0.010	−1.57	−0.110
(47.7 kg.)	Heart	0.319	0.535	0.339	0.205	0.544	−0.009 ± 0.019	−0.19	−0.013
B	Round	0.225	0.430	0.304	0.215	0.519	−0.089 ± 0.025	−1.69	−0.118
(52.7 kg.)	Heart	0.353	0.568	0.379	0.178	0.557	+0.011 ± 0.032	+0.21	+0.015
C	Round	0.229	0.434	0.293	0.167	0.460	−0.026 ± 0.009	−0.49	−0.034
(53.2 kg.)	Heart	0.359	0.574	0.380	0.210	0.590	−0.016 ± 0.016	−0.30	−0.021
D [4]	Round	0.236	0.509	0.316	0.219	0.535	−0.026 ± 0.017	−0.47	−0.033
(55.2 kg.)	Heart	0.369	0.652	0.403	0.226	0.629	+0.023 ± 0.010	+0.42	+0.029
All subjects	Round	0.223	0.445	0.307	0.192	0.499	−0.054 ± 0.009	−1.03	−0.072
(52.3 kg.)	Heart	0.350	0.582	0.375	0.205	0.580	+0.002 ± 0.010	+0.04	+0.003

[1] Average of 8 experimental days, except for subject B in beef heart period, when 7 days were used.

[2] Collagen furnished 8.58 per cent of total nitrogen; elastin 0.36 per cent.

[3] Collagen furnished 18.90 per cent of total nitrogen; elastin 0.39 per cent.

[4] Daily diet included 0.35 gm. nitrogen and 0.068 gm. phosphorus from psyllium seed.

SUMMARY

Four college women seemingly used the nitrogen of beef heart at least as well as that from beef round as indicated by practically identical coefficients of digestibility and losses of urinary nitrogen. A higher nitrogen balance for heart may probably be explained by a somewhat higher nitrogen intake.

Differences in utilization of phosphorus from the two meats, which were also in favor of beef heart, were not great and probably were also due to level in the diet. The heart contained more connective tissue than the round, but, as the balances for both nitrogen and phosphorus were slightly higher for this meat, it apparently did not prevent good usage of these elements.

Daily variations in the nitrogen and phosphorus metabolism of an individual subject and differences between subjects were often greater than the differences in availability of these elements from the two meats.

LITERATURE CITED

LONG, Z., AND M. S. PITTMAN 1935 Utilization of meat by human subjects. II. The utilization of the nitrogen and phosphorus of round and liver of beef. J. Nutrition, vol. 9, p. 677.

MITCHELL, H. H., AND JESSIE R. BEADLES 1927 The protein value in nutrition of beef liver, beef heart, and beef kidney. J. Biol. Chem., vol. 61, p. 429.

PITTMAN, M. S. 1932 The utilization by human subjects of the nitrogen, calcium, and phosphorus of the navy bean (Phaseolus vulgaris) with and without a supplement of cystine. J. Nutrition, vol. 5, p. 227.

SHERMAN, H. C. 1932 Chemistry of food and nutrition, 4th ed. The Macmillan Company, New York.

THE NUTRITIVE VALUE OF FUNGI

II. THE VITAMIN B, G AND B_4 CONTENT OF THE MYCELIUM OF ASPERGILLUS SYDOWI [1]

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FOUR FIGURES

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Although yeast has been widely used for several decades as a source of vitamin B, the closely related filamentous fungi belonging to the genera Aspergillus and Penicillium have not been examined for this substance until very recently.

Takata ('29) tested the dried mycelium of A. oryzae as a source of vitamin B for young growing albino rats. He concluded that the dried mycelium had about one-fifth the amount of the growth-promoting vitamin B possessed by dried brewer's yeast. Takahashi ('29) grew A. oryzae through many transfers on a modified Pfeffer's solution to exhaust all the vitamin B, and then grew the mold in larger quantities on the same medium. An extract of the mold caused good growth of pigeons and rats, and protected them from polyneuritis. Fawns and Jung ('33) reported that rats were cured of beriberi when a black mold developed spontaneously on their basal vitamin B-free diet. When no mold had formed on the basal diet the animals lost weight.

[1] The authors are indebted to Professors Hart and Elvehjem for advice regarding the technic which they have developed in the use of the chick for vitamins B, G and B_4 assay. They are also indebted to Dr. O. L. Kline for assistance in carrying out certain phases of the assay.

This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

In a previous report from this laboratory (Skinner, Peterson and Steenbock, '33) it was shown that the dried mycelium of A. fischeri and A. sydowi, when fed at a 20 per cent level, contained sufficient vitamin B to promote good growth of young rats and to afford complete protection against polyneuritis. Animals fed at a 5 per cent level failed to grow, and eventually became polyneuritic.

The present work was first undertaken to determine more accurately the content of the vitamin B complex in mold mycelium. Later, it was decided to determine as well the presence of various members of the vitamin B complex, availing ourselves of the use of the chick as the experimental animal.

EXPERIMENTAL

Preparation of mold mycelium. The mold used in these experiments was Aspergillus sydowi, one of the species used in our previous work (Skinner, Peterson and Steenbock, '33). It was grown under aseptic conditions on a sterilized medium of the following composition: 10 gm. NH_4NO_3, 6.8 gm. KH_2PO_4, 5 gm. $MgSO_4.7H_2O$, 0.16 gm. $FeCl_3.6H_2O$, 0.05 gm. $ZnSO_4.7H_2O$, and 100 gm. glucose, made up to 1 liter with tap water (Peterson, Pruess, Gorcica and Greene, '33). On this medium any vitamin found in the mycelium may be considered as having been synthesized by the organism. The mold was allowed to grow for 10 days. The mold mycelium was then removed, washed with cold water under the tap, squeezed lightly by hand to free it from water, packed into cans, frozen and stored at a temperature of 5°F. It was removed from cold storage as needed, ground to a fine paste and incorporated wet into the ration.

The rations for both rats and chickens, made up in quantities for 1 week's feeding, were kept in tightly-closed containers which were stored in an electric refrigerator. Because of the fact that growth of mold often took place on the mixed rations left in the cages overnight, especially on those with a high moisture content, the unconsumed portions were generally discarded each morning.

Composition of rat rations. The basal ration used with rats had the following composition: cooked cornstarch, 76 parts; acid-extracted casein, 18 parts; salt mixture no. 40 (Steenbock and Nelson, '23), 4 parts; and cod liver oil, 2 parts. Mold was added to this at levels of 10 and 20 per cent calculated on the dried basis, the amount of starch being reduced correspondingly. The ration therefore varied in water content according to the amount of mold added. The mold itself had a fairly constant water content of 75 per cent, with the exception of the batch which was fed for the first 3 weeks to the group getting mold at a 20 per cent level.

Mold as a source of the vitamin B complex for rats. Young male rats weighing from 50 to 60 gm. were used in this experiment. Each rat was placed in an individual cage with a screen bottom and screen sides. Each animal had his own feed cup in which a weighed portion of ration was placed each day, after any uneaten portion was discarded.

The group getting 20 per cent mold as the sole source of vitamin B was used as a control in another experiment and was limited to an intake of 537 gm. (dry basis) per rat over the 10 weeks period. This represents 107 gm. of dry mold eaten during the experiment, or an average intake of 1.53 gm. of mold per rat per day.

The growth curve (average of six) of this group is given in figure 1. The growth secured was excellent, the average gain of the group in 10 weeks was 139 gm. The break in the first part of the curve is due to limited consumption because of the high water content of the ration during the second week. The animals appeared to be in the best of health during the experiment. The portions of ration weighed out in the cups were completely consumed by each animal throughout the experiment, and the animals always seemed exceedingly hungry. There is little doubt that the animals would have eaten considerably more food if it had been given to them. The growth curve of this group represents the efficiency with which the ration was utilized rather than the greatest possible growth on the ration.

The group ate 96.7 gm. of casein and 38.7 gm. mold protein,[2] or 135.4 gm. of total protein. Thus the average gain in weight represents a gain of 1.03 gm. per gram of total protein consumed.

The group getting 10 per cent mold as sole source of vitamin B was not limited in consumption, except in so far as was necessary to keep the intake of the entire group approximately equal. The total intake in 10 weeks was 522 gm. The group made an average gain in weight of 130 gm. The consumption of mold was 52.2 gm. (dry basis) per rat in 10 weeks, or an average of 0.75 gm. per rat per day. Toward the end of the period three of the six rats showed a slightly decreased

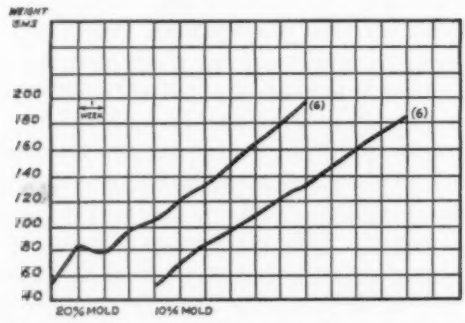

Fig. 1 Mold mycelium as a source of the vitamin B 'complex' for rats. The figures in parentheses indicate the number of individuals represented in each curve.

consumption of food. Except for this slight loss in appetite, none of the animals gave any signs of paralysis or any other disorder.

From the results obtained with these two groups, we may conclude that 20 per cent of mold (dry basis) in the ration, yielding 1.5 gm. of mold daily, furnished enough vitamin B to support good growth in young rats. Mold fed at a 10 per cent level yielding 0.75 gm. per day was almost as effective in promoting growth, but did not supply quite as great a stimulus to the appetite.

[2] Mold protein as calculated from $N \times 6.25$. This is known to be too high a figure, since much of the mold nitrogen is non-protein in character (Gorcica, Peterson and Steenbock, '34).

Consumption of chick rations. The technic and the rations used in demonstrating the presence of various members of the vitamin B complex were those worked out by members of this department (Kline, Keenan, Elvehjem and Hart, '32; Keenan, Kline, Elvehjem, Hart and Halpin, '33).

The basal ration used for the determination of vitamin B (B_1) contained the following: yellow corn, 58 parts; wheat middlings, 25 parts; casein, 12 parts; NaCl, 1 part; $CaCO_3$, 2 parts; cod liver oil, 2 parts. This ration, however, contains plenty of vitamin B, because it supports normal growth; on it the chicks may attain a weight of 300 gm. at the end of 6 weeks. To make it deficient in vitamin B, the yellow corn, wheat middlings, casein and salt were autoclaved at 120° and 15 pounds pressure for 5 hours. Four per cent of yeast which had been autoclaved for 5 hours at 15 pounds pressure was then added to supply an adequate amount of B_2(G), since the amount of G in the mold was not known. The ration treated in this manner has been designated as ration 241A.

A basal ration suitable for testing for vitamin G was prepared by heating the yellow corn, wheat middlings, casein and salt of the above basal ration at 100° for 144 hours in the dry state. This ration has been designated as ration 240H.

Ration 441A was used as the basal ration for determining vitamin B_4. This ration had the following composition: cooked cornstarch, 53.5 parts; casein autoclaved 5 hours at 15 pounds, 24.0 parts; salts, 2.5 parts; yeast, 8.0 parts; liver residue autoclaved 10 hours at 15 pounds, 10.0 parts; cod liver oil, 2 parts. Chicks on this ration develop a paralysis typical of avitaminosis B_4, which can be prevented by dried liver added at an 18 per cent level.

To introduce the mold into the basal rations, the frozen mold was ground to a fine paste, then mixed in while still very cold. The complete rations were packed into containers with tightly-fitting lids and stored in a refrigerator. They were made up fresh each week.

The chicks used for the experiment were day-old White Leghorns with an initial weight of 30 to 35 gm. obtained from the

University Poultry Department. They were placed in groups of ten in cages with screen bottoms. At the end of the first week, the number was reduced to seven in a group.

Mold as a source of vitamin B for chicks. On the basal autoclaved ration (241A) all of the chicks developed polyneuritis in 7 to 9 days and died in from 8 to 12 days (fig. 2). On the basal ration supplemented with 5 per cent mold, all of the chicks developed polyneuritis in from 10 to 16 days and died in from 11 to 19 days.

When mold was added at a level of 10 per cent, only one chick in the group developed polyneuritis. The six survivors

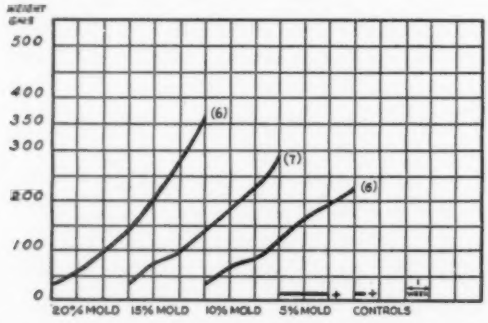

Fig. 2 Mold mycelium as a source of vitamin B for chicks. The numbers in parentheses indicate the number of individuals represented in each curve. The heavy horizontal lines followed by crosses indicate the length of life of the chicks on the rations concerned.

grew throughout the period, attaining an average weight of 218 gm. at the end of 6 weeks. This is not normal growth.

At a level of 15 per cent the mold completely prevented the appearance of polyneuritis. The average weight of the group at the end of 6 weeks was 296 gm. At a level of 20 per cent the mold completely prevented polyneuritis and in addition promoted better growth than at any of the lower levels. The chicks in this group attained an average weight of 362 gm. in 6 weeks, which is considered normal for this age.

Thus it is seen that, while 10 per cent of mold in the ration is sufficient to prevent polyneuritis, higher levels improve the growth performance.

Mold as a source of vitamin G for chicks. The growth curves of the chicks in this experiment are shown in figure 3.

The chicks in the negative control group (ration 240H) all developed pellagra in 2 to 4 weeks. The most obvious symptoms were the large black ulcers at the corners of the mouth, and, somewhat later, the appearance of a sticky exudate about the eyes. In many cases the eyelids adhered to one another and remained completely closed. At the end of 5 weeks all of the chicks still alive had the above severe symptoms of pellagra and were therefore discarded.

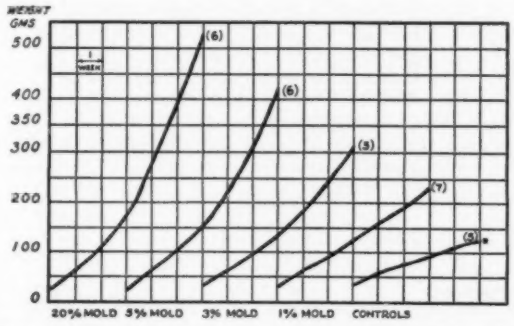

Fig. 3 Mold mycelium as a source of vitamin G for chicks. The numbers in parentheses indicate the number of individuals represented in each curve. The star at the end of the curve indicates that all members of the group had severe pellagra and were therefore discarded at that point.

The addition of mold at a 20 per cent level caused an exceptionally rapid growth—the group reached an average weight of 528 gm. in 6 weeks. When 5 per cent of mold was added to the basal ration, the chicks grew very well, reaching an average weight of 417 gm. in 6 weeks. They did not develop any symptoms of pellagra. When the level of mold was reduced to 3 per cent the chicks still grew fairly well, reaching an average weight of 306 gm. in 6 weeks. This level of mold still furnished complete protection against pellagra. At a 1 per cent level the mold still gave the chicks complete protection against pellagra, but the ration no longer supported good growth.

Mold as a source of vitamin B_4 for chicks. The growth curve of the group of chicks serving as negative controls on the basal ration is shown in figure 4. This group did not

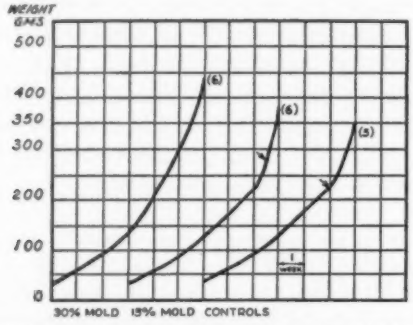

Fig. 4 Mold mycelium as a source of vitamin B_4 for chicks. The numbers in parentheses indicate the number of individuals represented in each curve. The arrows indicate the time of appearance of severe symptoms of paralysis.

TABLE 1
Summary

GROUP	RATION	AVERAGE WEEKLY WEIGHT OF CHICKS IN EACH GROUP							
		Start	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Number of survivors
1	R241A (low B)	33	All dead of polyneuritis in 12 days						
2	R241A + 5% mold	35	43	45	All dead of polyneuritis in 19 days				
3	R241A + 10% mold	35	63	79	116	163	190	218	6
4	R241A + 15% mold	35	69	95	140	183	219	296	7
5	R241A + 20% mold	31	56	98	143	201	271	362	6
6	R240H (low G)	33	55	69	90	108	123	—[1]	5
7	R240H + 1% mold	35	61	89	129	156	181	226	7
8	R240H + 3% mold	35	67	94	135	182	246	306	5
9	R240H + 5% mold	35	63	97	151	217	308	417	6
10	R240H + 20% mold	34	60	105	173	276	389	528	6
11	R441A (low B_4)	35	56	90	136	181	267	351	5
12	R441A + 15% mold	34	53	79	119	166	266	383	6
13	R441A + 30% mold	34	55	91	140	220	312	419	6

[1] All the chicks in the group showed severe symptoms of pellagra and were therefore discarded.

behave as chicks on ration 441A usually do, and only two of the chicks developed paralysis. This may be attributed to the fact that an error was made in preparing one of the

batches of ration. During the third week of the experiment the basal ration was made up with 10 per cent liver that had been autoclaved only half the usual time.

When the basal ration was supplemented by 15 per cent mold, the growth was slightly improved, but one of the chicks developed a well-defined case of paralysis. In view of the unusually good performance of the negative control group, the appearance of paralysis on the 15 per cent mold ration probably means that this level is quite inadequate as a source of vitamin B_4.

The group getting ration 441A supplemented with 30 per cent mold made a rather slow start, but grew very rapidly during the final weeks of the experiment. None of the chicks in this group showed any signs of paralysis at any time during the 6 weeks period. As shown in table 1, this group attained an average weight of 419 gm. in 6 weeks. It seems quite safe to assume that 30 per cent of mold in the ration gives adequate protection against vitamin B_4 deficiency.

SUMMARY

The addition of 10 per cent of the mycelium of A. sydowi to a ration low in vitamin B (complex) furnished enough of the vitamin to support good growth in rats. Better growth and performance was secured on 20 per cent of the mold.

The mold contained enough vitamin B(B_1) to protect chicks against polyneuritis when it was fed at a 10 per cent level.

Symptoms of pellagra were prevented in chicks on a ration low in vitamin G by the addition of 1 per cent of mold. To secure good growth of chicks it was necessary to add 3 per cent of mold.

Mold added at a level of 30 per cent to a ration deficient in vitamin B_4 completely prevented the onset of paralysis.

LITERATURE CITED

FAWNS, H. T., AND A. JUNG 1933 The mineral content of the skin of rats suffering from beri-beri, with investigations on the formation of vitamin B_1 by molds. Biochem. J., vol. 27, p. 918.

GORCICA, H. J., W. H. PETERSON AND H. STEENBOCK 1934 The chemistry of mold tissue. V. Fractionation of the nitrogen in the mycelium of Aspergillus fischeri. Biochem. J., vol. 28, p. 504.

KEENAN, J. A., O. L. KLINE, C. A. ELVEHJEM, E. B. HART AND J. G. HALPIN 1933 New nutritional factors required by the chick. J. Biol. Chem., vol. 103, p. 671.

KLINE, O. L., J. A. KEENAN, C. A. ELVEHJEM AND E. B. HART 1932 The use of the chick in vitamin B_1 and B_2 studies. J. Biol. Chem., vol. 99, p. 295.

PETERSON, W. H., L. M. PRUESS, H. J. GORCICA AND H. C. GREENE 1933 Large-scale laboratory cultivation of molds. Ind. Eng. Chem., vol. 25, p. 213.

SKINNER, J. T., W. H. PETERSON AND H. STEENBOCK 1933 Nährwert von Schimmelpilzmycel. Biochem. Z., Bd. 267, S. 169.

STEENBOCK, H., AND E. M. NELSON 1923 Fat-soluble vitamine. XIII. Light in its relation to ophthalmia and growth. J. Biol. Chem., vol. 56, p. 355.

TAKAHASHI, E., AND R. K. SHOKU 1929 Synthesis of vitamin B by Aspergillus oryzae. J. Agr. Chem. Soc. Japan, vol. 5, p. 697.

TAKATA, R. 1929 An undertaking on the utilization of microorganisms for human food materials. IV. Vitamins in the mycelium of Aspergillus oryzae. J. Soc. Chem. Ind. Japan, vol. 32, p. 157B.

THE NUTRITIVE VALUE OF FUNGI

III. THE GROWTH OF RATS ON SUPPLEMENTED AND UNSUPPLEMENTED MOLD PROTEINS [1]

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FIVE FIGURES

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It has long been known that some of the filamentous fungi, especially the members of the genera, Aspergillus and Penicillium, are able to synthesize their cell proteins using inorganic salts as the sole source of nitrogen. This ability of converting inorganic nitrogen into protein nitrogen has led to speculations on the possibility of using mold proteins for food.

In Germany attempts were made to produce cattle fodder of increased protein content from straw by the use of a species of Aspergillus (Pringsheim and Lichtenstein, '20). In Japan the availability of mold proteins to albino rats was studied with the species which is used extensively in Japan in the manufacture of Koji-sauce, Aspergillus oryzae (Takata, '29). The protein of this mold was shown to be incomplete, and the addition of cystine did not correct the deficiency. Skinner ('34) recently reported that the proteins synthesized by Penicillium from an inorganic-glucose medium contained at least traces of the essential amino acids, but not in sufficient amounts to permit much growth in rats at a protein level of 9 per cent. At an 18 per cent level growth was improved, but still was not normal. When the ration containing 9 per

[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

cent of mold protein was supplemented with 0.25 per cent cystine, growth was improved, but no figures were given as to the extent of the improvement.

In all problems in which the composition of molds is a factor, it is necessary to keep in mind the extreme variations with species and the variability in each species when grown on different media and under different conditions. Thus, in a survey of the composition of various molds carried out in this laboratory (Pruess, Eichinger and Peterson, '34), it was found that under identical conditions the crude protein ($N \times 6.25$) content of a number of molds varied from 13.7 per cent to 43.7 per cent. Also, when grown on a glucose-inorganic salts medium, P. chrysogenum contained 43.7 per cent crude protein, while on a glucose-malt sprouts medium it contained only 15 per cent. A. oryzae which contained 33.7 per cent crude protein when grown on glucose-inorganic salts, increased this figure on glucose-malt sprouts to 36.3 per cent, although the average protein content of twenty-four molds was 30 per cent lower on the malt-sprouts medium than on the inorganic salts medium. The amount of lipid, sterol and carbohydrate in these molds also showed great variations.

There is, moreover, considerable evidence that even pure strains of molds will change their metabolism with long-continued propagation on stock agar media. A classical example of this phenomenon was the discovery by Wehmer (cited in Bernhauer, '32) that an Aspergillus which originally had produced fumaric acid subsequently degenerated, and completely lost the ability to form this acid.

In the light of the above considerations, there need be no contradiction between the findings of Takata ('29) and Skinner ('34), one of whom found that cystine did not remedy the deficiency of mold protein, while the other reported that cystine was the primary limiting amino-acid. The mere fact that the two investigators used different species of mold might well explain the discrepancy.

In the course of experiments previously carried out in this laboratory (Skinner, Peterson and Steenbock, '33), it was

found that the proteins of four different molds were incapable of promoting growth of rats when fed at the 18 per cent level. When mold replaced part of the starch in a complete ration, the growth rate was reduced, but when mold was added to a ration containing only 4 per cent casein, growth was improved. The addition of small quantities of casein or yeast to rations containing 18 per cent mold protein greatly improved growth.

In making further studies of mold protein for the purpose of ascertaining the nature of the deficiencies, it was decided to concentrate attention on a single mold. The mold chosen was Aspergillus sydowi, a species which grows vigorously on a liquid synthetic medium.

EXPERIMENTAL

Preparation of mold mycelium. The mycelium used in these experiments was from the same strain of Aspergillus sydowi as that used in previous work in this laboratory (Skinner, Peterson and Steenbock, '33). It was grown on a glucose-inorganic salts medium of the following composition: 10 gm. NH_4NO_3, 6.8 gm. KH_2PO_4, 5 gm. $MgSO_4.7H_2O$, 0.16 gm. $FeCl_3.6H_2O$, 0.05 gm. $ZnSO_4.7H_2O$, and 100 gm. glucose made up to 1 liter with tap water. This medium was acidified with 1 cc. of 85 per cent H_3PO_4 per liter, placed in pans (10 liters per pan) in sterilizer-incubators (Peterson, Pruess, Gorcica and Greene, '33), and steamed at 100° for 8 hours. After the steam was turned off, a stream of sterile air was forced into each fermenter.

When the fermenters had cooled to room temperature, they were inoculated through the side openings with 100 cc. of a spore suspension of A. sydowi for each pan of medium. The temperature of the room was maintained at 30° for the incubation period, 10 days.

At the time of harvest the mold pads were rinsed once with cold water, pressed out by hand, and then stored in cans in a refrigerator at 5°F. Later, they were removed from the cans with a steel chopper, ground immediately to a fine paste

and mixed with the other ingredients of the ration as needed. The rations were kept in tightly-covered containers in an electric refrigerator, from which they were removed only at feeding time.

Composition of rations. The basal ration had the following composition: 50 parts mold (dry basis), 44 parts cooked cornstarch, 4 parts mineral salts mixture no. 40 (Steenbock and Nelson, '23), and 2 parts cod liver oil. Mold was the only source of nitrogen in this ration. When protein supplements were added to the basal ration, a corresponding amount of starch was taken out. From previous experiments it was known that 20 per cent mold in the ration supplied ample amounts of the vitamin B complex.

Control of consumption. The animals used were young vigorous male rats of 50 to 60 gm. weight. Six rats were started in each group. Each animal was placed in an individual cage with a screen bottom. Each morning the unconsumed ration was weighed and discarded and a fresh portion was weighed out.

The intake of all the animals which were given a 5 per cent protein supplement was held for the first 4 weeks to that of the control group, for the remaining 6 weeks to that of the group getting 5 per cent skim-milk protein. The control group had the lowest intake throughout the experiment, but the rapid decrease in consumption by this group in the fifth and sixth weeks, together with the death of animals in the group, made it inadvisable to hold down the intake of the other groups to this level. Accordingly, for the last 6 weeks the intake of the others was controlled by that of the next limiting group, which was the group getting 5 per cent skim-milk protein.

Within the limiting groups the intake of the animals was held, in general, to that of the rat that ate the least. When the intake of one animal in the group fell below that of the preceding week, the other animals were not restricted to this level, but were held to that of the preceding week. The consumption of ration per animal per week by those getting 5

per cent protein supplements was as follows: 37, 32, 48, 44, 48, 56, 60, 64, 68, 70 gm., total, 527 gm.

When the groups getting 2½ per cent protein supplements were started, it was planned to limit the intake of each animal to 527 gm. over a 10-week period while keeping the weekly intakes of all animals equal. This plan was abandoned at the end of the seventh week when the intakes of all the animals began to decline, and during the last 3 weeks consumption was equalized only within groups. The weekly intake per animal was as follows: 40, 40, 42, 47, 50, 51, 56, 59, 65, 70 gm., total 520 gm. In cases where the intake varied from that given above, it will be referred to in the discussion.

Growth on protein supplements. For control purposes one group was given the basal ration unsupplemented, so that the mold in the ration was the sole source of nitrogen. The average growth curve of the group is shown in figure 1. All of the animals on the control ration died in from 4 to 7 weeks, but they all gained in weight before death—the average gain in weight of the group at time of death was 16 gm. Since the mold in the ration was known to supply ample quantities of the vitamin B complex (preceding paper), it is evident that the proteins of A. sydowi did not support life when the mold was fed at a level of 50 per cent, equivalent to 18 per cent of crude protein (N $\times$ 6.25).

The materials used as protein supplements were casein, dried skim-milk powder, whole wheat, corn gluten feed, whole corn, dry powdered egg white, soy bean meal, gelatin and yeast.

Casein was given as a protein supplement to three groups of rats at 10, 5 and 2½ per cent levels (fig. 1). Although the rats were limited to the same food intake as the control group, the average gain in weight of the first group was 131 gm., of the second group 101 gm., and of the third group 64 gm. The addition of as little as 2½ per cent casein markedly improved the ration. The intake, average gain in weight, and the gain in weight per gram of protein consumed are given in table 1.

Since cystine is the limiting amino-acid in casein (Osborne and Mendel, '15), it appears improbable that the additional cystine supplied by the casein at a 2½ per cent level prevented the early death of the animals and supported the greatly increased growth noted. It seems more likely that the factor responsible for this pronounced effect at such a low level must be some rather abundant constituent of the casein.

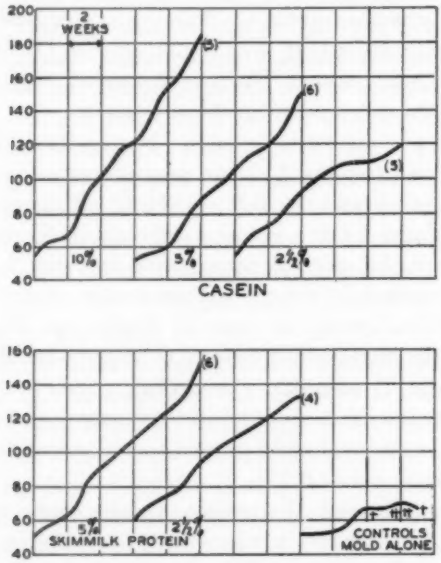

Fig. 1 Average growth curves of rats on mold as the sole source of protein and with supplements of casein and skim milk protein at various levels. The figures in parentheses indicate the number of individuals represented by the curve. The crosses beneath the curve representing the control group indicate the death of animals in the group.

Dry skim-milk powder was fed at levels such that the rations contained 5 and 2½ per cent skim-milk protein. The average gain in weight (fig. 1) was almost identical with that on the corresponding levels of casein. This fact provides further evidence that the mold is probably not deficient in cystine, since if cystine were the factor supplied by the supplements,

TABLE 1

Growth of rats on mold mycelium supplemented by certain proteins

TYPE OF PROTEIN ADDED	CASEIN			DRIED SKIM-MILK		CORN GLUTEN FEED		WHOLE WHEAT		EGG WHITE	
Level of supplement, per cent	2½	5	10	2½	5	2½	5	2½	5	2½	5
Ration consumed, grams	504	537	537	508	537	520	537	520	537	501	463
Total protein consumed, grams	103	124	150	104	124	107	124	107	124	103	106
Supplementary protein consumed, grams	12.6	26.9	53.7	12.7	26.9	13.0	26.9	13.0	26.9	12.5	23.2
Number of survivors after 10 weeks[1]	5	6	5	4	6	6	6	5	4	5	5
Average gain in weight, grams	64	101	131	72	99	111	101	100	118	66	73
Gain in weight per gram of supplementary protein consumed, grams	5.1	3.8	2.5	5.7	3.7	8.5	3.8	7.7	4.4	5.3	3.2
Gain in weight per gram of total protein consumed, grams	0.62	0.81	0.87	0.69	0.80	1.04	0.81	0.94	0.95	0.64	0.69

[1] Number of original animals (six).

the proteins of skim milk, due to their content of lactalbumin, should be considerably more effective than casein in promoting growth when added to mold.

The proteins of whole wheat were fed as a supplement at levels of 5, 2½ and ½ per cent. The average gains on the 5 per cent level were 118 gm. and on the 2½ per cent level 100 gm.

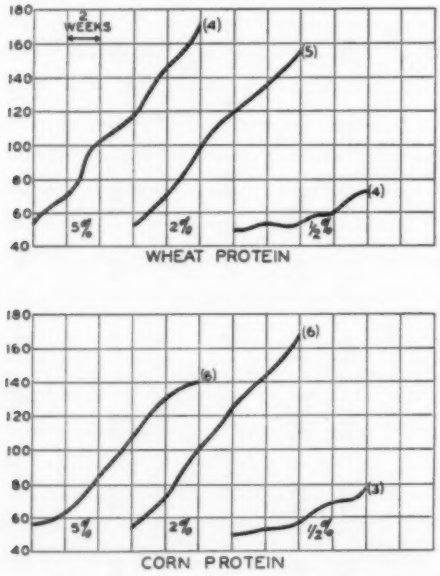

Fig. 2 Average growth curves of rats on mold supplemented by wheat and corn proteins at various levels. The figures in parentheses indicate the number of individuals represented by the curve.

(fig. 2). The animals getting ½ per cent wheat protein grew very slowly. At the end of 8 weeks the four survivors had gained on the average 23 gm. At this time two of the animals were given supplements of amino-acids. These animals will be discussed later.

Corn protein in the form of corn gluten feed (from Pennick & Ford, Cedar Rapids, Iowa) was fed at levels of 5 and 2½

per cent. Both groups grew well; the group on the lower level made an average gain of 111 gm., which is the best record made by any of the groups getting a 2½ per cent protein supplement. As shown in table 1, this group gained 1.04 gm. per gram of crude protein consumed. Inasmuch as the mold nitrogen is not more than two-thirds protein nitrogen (Gorcica, Peterson and Steenbock, '34), the protein of the ration was quite efficiently utilized.

One group was fed the proteins of whole corn at a level of ½ per cent, and in 8 weeks the three survivors had gained an average of 29 gm. The growth curves of this group are shown in figure 4.

Since Mitchell and Smuts have shown ('32) that the proteins of corn are deficient in lysine and tryptophane, and since the addition of only 2½ per cent of corn protein to the mold ration promotes good growth, the mold proteins are apparently not markedly deficient in either lysine or tryptophane.

Dried powdered egg white (Mallinckrodt's "Egg Albumin-Soluble-Impalpable powder") was fed at levels of 5 and 2½ per cent. The average total intake of the group getting 5 per cent egg white was only 463 gm., while the average gain of the group was only 73 gm. This was the smallest gain of any group getting a 5 per cent supplement of protein (except those getting gelatin), but it was undoubtedly due to the low intake. At the 2½ per cent level the intake was 501 gm. and the average gain 66 gm.

The growth on the ration supplemented with egg white was so much poorer than had been expected that it was decided to determine whether the egg white used was a raw or denatured preparation. The following ration was used: cooked cornstarch, 70; egg white, 18; mineral salts mixture no. 40, 4; agar, 2; cod liver oil, 2. Three rats were fed this ration made up with the same egg white as before, while three others were given this egg white after it had been cooked in an autoclave at 15 pounds pressure for 20 minutes. The growth curves of these animals are given in figure 3.

The animals getting raw egg white each consumed 400 gm. of ration in 10 weeks. Their average gain during that time was 23 gm. The animals began to lose hair after having been on the ration 5 weeks, and at the end of 10 weeks they had lost the hair over almost the entire body. Some of these symptoms were the same as those described by Parsons and Kelly ('33) in their paper on the toxicity of egg white to rats. The group getting autoclaved egg white made an average gain

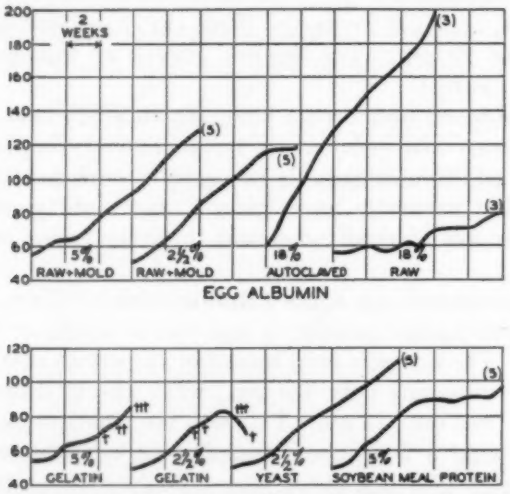

Fig. 3 Average growth of rats on mold supplemented with various proteins. Also the growth of rats on heated and unheated egg white as sole source of protein. Crosses indicate death of animals in the group.

of 140 gm. in 10 weeks on an intake of 633 gm. These animals lost no hair and were in excellent health throughout the experiment. On the evidence of this experiment, it seems very likely that the preparation used was raw egg white, which fact explains the poor results obtained with it as a supplement to the mold.

The supplemental value of soybean protein was also determined. One group was given soybean meal protein at a 5 per cent level. The meal was a preparation that had been heated

at 140° for ½ hour, and was known to be palatable to rats. The group made an average gain of 48 gm. in 10 weeks on an intake of 459 gm. (fig. 3). The intake of the group was declining rapidly during the last 2 weeks of the experiment.

Gelatin proved to be a very poor supplement. It was fed at two levels. When added at a 5 per cent level, the animals died in from 4 to 6 weeks, after making some growth which totalled 21 gm. at the time of death. When the ration contained 2½ per cent gelatin, five animals died in 3 to 6 weeks, while one lived for 9 weeks. The average gain in weight at the time of death was 27 gm. The growth curves of these two groups are shown in figure 3.

One group of rats was fed a supplement of yeast at a 5 per cent level, equivalent to 2½ per cent protein. The average gain of the group was 68 gm. on an intake of 537 gm. The average growth curve of this group is shown in figure 3. Yeast protein appears to have about the same supplemental value as casein or skim-milk powder.

Growth on amino-acid supplements. From the data given above it appeared that the mold protein was not deficient in lysine or tryptophane, nor markedly deficient in cystine, but suggested the possibility of a histidine deficiency. It was decided to try the addition of various amino-acids.

A group of rats was available that had been on a ration containing 18 per cent mold protein plus ½ per cent whole wheat protein or ½ per cent whole corn protein for 8 weeks. Of the surviving animals which had received the wheat supplements, two were continued on this ration as controls, and the other two were placed on a ration in which the wheat had been replaced by ½ per cent cystine plus 1 per cent tyrosine. The food intakes of the four rats were kept equal. The experiment was continued for 4 weeks. Of three rats which had received corn protein, one was continued as a control, while the other two were given ½ per cent cystine and 1 per cent tyrosine instead of the corn. The addition of cystine and tyrosine failed to improve growth (fig. 4).

A group of six rats, 85 to 103 gm. in weight, was started on a ration containing 18 per cent mold protein. After a rapid initial loss in weight, the animals began to grow very slowly, but at the end of 8 weeks their average weight was only 7 gm. more than when they were put on the experiment. Two of these animals were given a supplement of ½ per cent cystine, two others were given 1 per cent tyrosine, and two were given ½ per cent cystine plus 1 per cent tyrosine. In no case did the

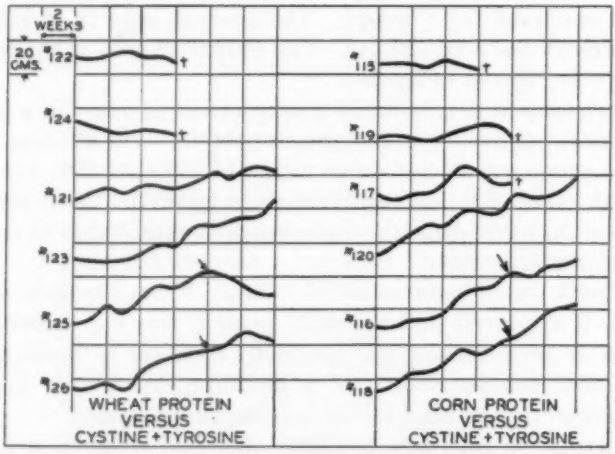

Fig. 4 Comparison of wheat and corn protein with cystine and tyrosine as supplements to mold protein. Arrows indicate point at which 0.5 per cent corn or wheat proteins were replaced by 0.5 per cent cystine plus 1 per cent tyrosine.

addition of these amino-acids cause any decided improvement in growth. It therefore seems quite definitely established that the addition of cystine and tyrosine to our mold did not improve its growth-promoting power.

To test the effect of supplementing mold protein with histidine, a group of seven rats was started of which three as controls received 18 per cent mold protein, while the others were given in addition ½ per cent histidine. As shown in figure 5, three of the rats getting histidine grew more rapidly than the three control rats, but died in 6 weeks or less, while no. 153,

which grew at about the same rate as the control animals, lived the longest. At the end of 7 weeks, it was obvious that the addition of histidine to the mold ration would not support continued growth and the group was therefore discarded.

It is evident, that other combinations of amino-acids or supplements must be tried before the deficiencies of the mold mycelium can be determined.

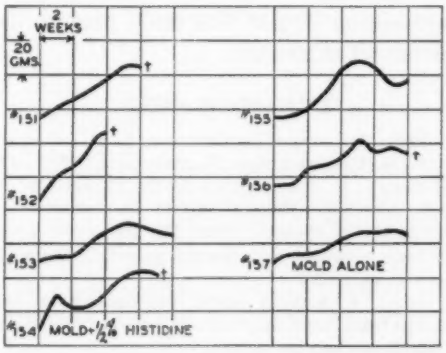

Fig. 5 Supplementary value of histidine to mold protein.

SUMMARY

The nutritive value of the proteins of Aspergillus sydowi, when produced on a synthetic medium (inorganic salts-glucose), was determined. The mold mycelium was fed as the sole source of protein or supplemented with various amounts of other proteins or with certain amino-acids.

The proteins of A. sydowi were found insufficient to promote more than a slight amount of growth or to sustain life in young rats for a period longer than 7 to 9 weeks.

The most effective supplements were found to be the proteins of whole wheat and corn gluten feed. When these were added to the basal ration at either a 2½ or a 5 per cent level, the resulting ration supported very good growth.

Supplementing the mold protein with casein, skim-milk protein, egg white and yeast protein at levels of 2½ and 5 per cent resulted in good growth, but growth was better at the higher level.

Supplementation with gelatin at 2½ or 5 per cent levels did not improve the growth-promoting power of the mold protein.

The addition of ½ per cent cystine, ½ per cent histidine, 1 per cent tyrosine, or ½ per cent cystine plus 1 per cent tyrosine, to a ration containing 18 per cent mold protein did not result in any improvement in growth.

LITERATURE CITED

BERNHAUER, K. 1932 Die oxydativen Gärungen, S. 63. J. Springer, Berlin.

GORCICA, H. J., W. H. PETERSON AND H. STEENBOCK 1934 The chemistry of mould tissue. V. Fractionation of the nitrogen in the mycelium of Aspergillus fischeri. Biochem. J., vol. 28, p. 504.

MITCHELL, H. H., AND D. B. SMUTS 1932 The amino acid deficiencies of beef, wheat, corn, oats, and soybeans for growth in the white rat. J. Biol. Chem., vol. 95, p. 263.

OSBORNE, T. B., AND L. B. MENDEL 1915 The comparative nutritive value of certain proteins in growth, and the problem of the protein minimum. J. Biol. Chem., vol. 20, p. 351.

PARSONS, H. T., AND E. KELLY 1933 The effect of heating egg white on certain characteristic pellagra-like manifestations produced in rats by its dietary use. Am. J. Physiol., vol. 104, p. 150.

PETERSON, W. H., L. M. PRUESS, H. J. GORCICA AND H. C. GREENE 1933 Large-scale laboratory cultivation of molds. Ind. Eng. Chem., vol. 25, p. 213.

PRINGSHEIM, H., AND S. LICHTENSTEIN 1920 Versuche zur Anreicherung von Kraftstroh mit Pilzeiweiss. Cellulosechemie, Bd. 1, S. 29.

PRUESS, L. M., E. C. EICHINGER AND W. H. PETERSON 1934 The chemistry of mold tissue. III. Composition of certain molds with special reference to the lipid content. Zentr. Bakt. Parasitenk., II Abt., Bd. 89, S. 370.

SKINNER, C. E. 1934 The synthesis of aromatic amino acids from inorganic nitrogen by molds and the value of mold proteins in diets. J. Bact., vol. 28, p. 95.

SKINNER, J. T., W. H. PETERSON AND H. STEENBOCK 1933 Nährwert von Schimmelpilzmycel. Biochem. Z., Bd. 267, S. 169.

STEENBOCK, H., AND E. M. NELSON 1923 Fat-soluble vitamine. XIII. Light in its relation to ophthalmia and growth. J. Biol. Chem., vol. 56, p. 355.

TAKATA, R. 1929 An undertaking on the utilization of microorganisms for human food materials. II. The nutritive value of the protein in the mycelium of Aspergillus oryzae. J. Soc. Chem. Ind. Japan, vol. 32, p. 155B.

THE NUTRITIVE VALUE OF LACTOSE IN MAN

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ONE FIGURE

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Information on the nutritive value of lactose in man is very meager and only indirect evidence is available. Berglund and Folin ('22) showed in one case that after giving 100 gm. of lactose orally to a subject there was only a slight or insignificant rise in the blood sugar. This inability of lactose to produce a hyperglycemia could be explained either by a failure of, or delay in, its absorption from the alimentary tract or by its rapid combustion or storage. Higgins ('16) studied the effects of the ingestion of lactose on the respiratory quotient and oxygen consumption and found the respiratory quotient well above 1.00; in fact, even considerably higher than after the administration of an equivalent amount of glucose. Benedict and Carpenter ('18) also obtained in man a specific dynamic effect following lactose administration equal to or greater than that of glucose. More recently, McCullagh and McCullagh ('32) showed that lactose had an effect similar to glucose in lowering inorganic blood phosphorus and in decreasing its excretion in the urine. The exact significance of the inorganic blood phosphate changes after sugar ingestion is unknown, but the lowering of the phosphate curve has usually been looked upon as evidence of sugar utilization. The above investigators found, however, that galactose does not alter the blood inorganic phosphate or its urinary excretion, although it can be utilized by the body at least to a consider-

able extent. From the standpoint of absorption of lactose from the alimentary tract the early observations of Bourguelot and Troisier (1889) are interesting. They fed lactose to a diabetic subject and recovered in the urine two molecules of glucose for each of lactose ingested and no galactose was detected.

It can readily be seen that the information on lactose utilization from the nutrient standpoint in man is altogether inadequate. The data available is based on indirect evidence, the physiological significance of which is still uncertain.

PLAN OF STUDY AND METHODS

The glycemic response after lactose ingestion was studied in normal, obese, and diabetic subjects. The value of this was emphasized after a few subjects were studied, inasmuch as there was not only considerable variation in apparently normal cases, but a greater variation was noted when diabetic and obese subjects were compared with the normal group. Lactose was given to subjects (1.5 gm. per kilogram) in the morning in a fasting state. Venous blood and urine samples were collected hourly for 4 hours. Except in a few cases, the subjects were at rest, either lying or sitting during the period of study. If the urine specimen gave a positive reduction test, the fermentability of the sugar was determined. The Hartman-Shaffer method for blood sugar was used.

In a group of normal cases the blood inorganic phosphate changes after lactose ingestion were also studied. This work was started before the observations of McCullagh and McCullagh ('32) came to our attention and then it was decided to extend their observations on a few cases to a larger series.

RESULTS

Glycemic effect. As a basis for comparison, particularly from the standpoint of individual variation and average values, the blood sugar and urinary excretion after glucose ingestion was studied in twenty-six apparently normal cases. The average glycemic response is shown in figure 1. Con-

siderable variation was noted, but the average values form a reliable basis for comparison. The comparable results after lactose ingestion in eighteen normal young adults are shown also. Although 80 to 120 gm. of lactose were given in a single dose, only in one case was there flatulency and diarrhea. The

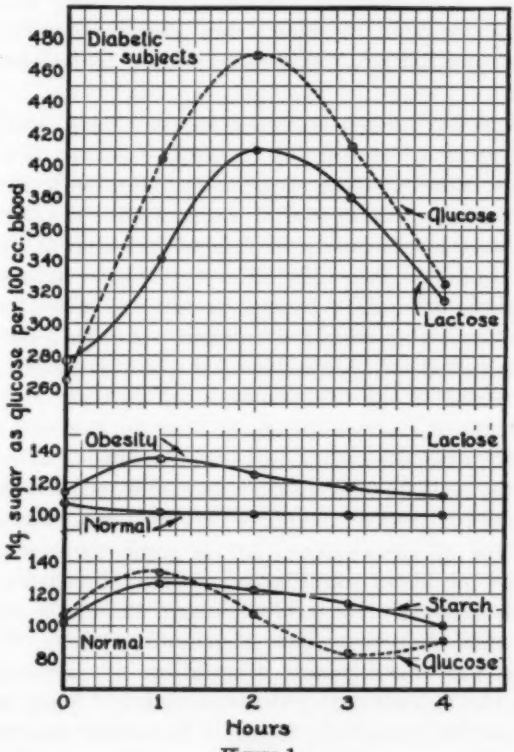

Figure 1

individual variations in the blood sugar values were again quite large, comparable to those obtained with glucose. In general, lactose ingestion resulted in no appreciable increase in blood sugar and the average values show quite clearly the lack of any hyperglycemic effect. The average values for the first, second, third and fourth hours after lactose are sur-

prisingly constant. The secondary hypoglycemic effect usually found at the time of the third hour after glucose ingestion, was much less, practically insignificant, after lactose. Glycosuria was much more frequent after lactose than after glucose administration, but the excreted sugar was nonfermentable in all cases studied and apparently was lactose that was assimilated as such from the intestinal tract, although the possibility of this having been galactose was not eliminated.

The average glycemic response in six normal adults after the ingestion of a comparable amount of starch is shown to compare the rate of sugar absorption after the hydrolysis of a polysaccharide. At the end of the first hour the blood sugar rise after starch was only a little less than that after glucose ingestion, but the hyperglycemia was more prolonged for starch and the secondary hypoglycemia was absent as in the case of lactose. Our results are consistent with those of Kjer ('24), who found that starch caused quite as great a hyperglycemia as did an equivalent amount of glucose.

The results of lactose tolerance tests in a group of obese subjects are also shown. The hourly averages after lactose ingestion in sixteen overweight cases showed a definite rise for the first and second hours. This rise was, however, not universal for all obese cases, for in some there was even a lowering of the blood sugar after lactose administration. In some obese cases the rise in blood sugar was of the magnitude found in mild diabetics after glucose ingestion. The effect of fasting and mechanical stimulation of peristalsis was studied in two subjects after the ingestion of 1.5 gm. powdered cellulose per kilogram body weight. In neither case was there any appreciable change in blood sugar. The rise of the blood sugar in the majority of obese cases indicated that in all probability there is at least partial assimilation of ingested lactose.

The effect of lactose ingestion in eight cases of moderately severe diabetes mellitus is also shown in figure 1. The marked increase in the blood sugar in the first, second, third and even

fourth hours after lactose administration is apparent. In all these cases the blood sugar increase over the fasting specimens was accounted for as fermentable sugar. In these subjects the equivalent amount of glucose was also administered and no very marked differences were obtained between the blood sugar curves after lactose or glucose. The peaks for the lactose and glucose curves were at approximately the same time indicating that there was no gross delay due to splitting of the lactose. In one case a control glycemia curve was obtained after the equivalent amount of powdered cellulose ingestion and little variation in the 4 hours after fasting was noted. It was of particular interest to note that, in spite of considerable excretion of 'extra sugar' in the urine, as much as 13.38 gm. for the third hour in one case, the urinary sugar in every instance was all fermentable. In other words, lactose or galactose was not excreted in the diabetic as it usually was in the normal individual after lactose ingestion. Bourquelot and Troisier had already pointed this out in one case in 1889, although their analytical methods were unrefined as compared with those of the present day. They fed lactose to a diabetic patient and recovered two molecules of glucose in the urine for each one of lactose fed. It would seem from these experiments that the diabetic not only assimilates lactose, but that he also completely converts, at least the excreted sugar, to glucose. This latter phenomenon is, to our knowledge, the only specific example of the ability of the diabetic to convert a substance to glucose which normally may be excreted in another form (lactose or galactose). This observation is of interest from the standpoint of the overproduction hypothesis as a causative factor in diabetes.

The recovery in the urine of lactose administered intravenously. The failure of the diabetic to excrete lactose raised the question of his ability to convert intravenously injected lactose. The lactosuria in the normal after lactose ingestion is undoubtedly due to the absorption of lactose as such from the bowel. There is no reason to believe that such an absorption should not also take place in the diabetic and its failure of being excreted points to its conversion or destruction.

Table 1 gives the amount of lactose recovered in the urine as non-fermentable sugar after a known amount of lactose was slowly injected as a 10 per cent solution. In five normal cases the average per cent recovered was 93.2, while in three obese cases 89.7 per cent was recovered. The small decrease in the recovery in the obese group is probably insignificant because of the greater dilution of the same amount of lactose in the

TABLE 1

The recovery in the urine of lactose administered intravenously

CASE	AMOUNT GIVEN	AMOUNT RECOVERED	PER CENT RECOVERED
		Normal	
	gm.	gm.	
A. E. K.	15.5	14.1	91
G. B.	14.2	14.0	98.5
G. W.	14.2	12.3	86.5
D. Mc.	14.0	13.8	98.7
B. W.	13.1	12.0	91.5
Average	14.2	13.2	93.2
		Obese	
A. W.	14.0	12.3	87.9
E. B.	15.0	14.0	93.4
E. W.	14.6	13.0	88.0
Average	14.4	13.1	89.7
		Diabetic	
M. F.	14.6	13.0	88.0 (with insulin)
M. F.	15.5	13.7	88.5 (without insulin)
G. W.	13.1	12.0	91.5
Average	14.3	12.9	89.3

blood and tissue fluids in these subjects as compared with normal individuals. In two diabetic subjects studied the recovery of excreted lactose was approximately the same as in the normal or obese groups. The injection of 30 units of insulin 45 minutes before the lactose injection in the case of M.F. had no effect on the lactose recovery. Apparently the diabetic subject has no greater power to destroy intravenously administered lactose than the normal individual. Consequently other factors must play a role in the absence of

lactosuria in the diabetic after lactose ingestion and a difference in the process of alimentation may be suspected.

The possibility of liver lactase in the human as has been found by Cajori ('34) in the liver of the dog must be kept in mind. This may account for 10 per cent of the lactose that was destroyed after intravenous administration.

The effect of lactose on the inorganic phosphate content of the blood. The effect of glucose administration on the inorganic phosphate content of the blood is well known. Our results shown in table 2 substantiate the findings of McCullagh

TABLE 2

The effect of 1.5 gm. lactose per kilogram orally on the inorganic phosphate content of whole blood. (Expressed as milligrams per 100 cc. of blood)

CASES	FASTING	FIRST HOUR	SECOND HOUR	THIRD HOUR	FOURTH HOUR
E. H.	4.1	3.3	2.9	2.9	3.1
F. H.	3.3	3.3	3.2	3.3	3.3
C. H.	2.9	2.9	2.6	2.9	3.3
M. Mc.	2.6	2.5	2.5	2.8	3.0
K. O.	2.9	3.0	2.9	3.0	3.0
M. H.	3.7	3.6	3.7	3.6	3.6
I. C.	3.6	2.9	3.0	3.1	3.3
E. P.	3.0	2.2	2.4	2.4	2.7
Average (8 cases)	3.3	3.0	2.9	3.0	3.2

and McCullagh ('32) that lactose lowers the blood inorganic phosphate, although in our series of eight normal subjects the average decrease was only 0.4 mg. per 100 cc. of blood. This small change must not necessarily be interpreted as indicating lactose utilization.

DISCUSSION

The average blood sugar values in normal individuals after lactose ingestion showed quite clearly the absence of any hyperglycemic effect. Such a failure to elevate the blood sugar might well be due to increased rate of utilization and storage or to delayed absorption and consequently offers no proof as to whether or not lactose is absorbed from the ali-

mentary tract. If the failure of hyperglycemia is due to delayed absorption from slow hydrolysis, then the rate of splitting of lactose must be very much slower than that of starch, for the latter causes a rise in blood sugar as quickly and nearly as great as obtained after glucose ingestion. Such slow splitting of lactose together with the rapid rate that it may pass through the small intestine (Hosoi, Alvarez, and Mann, '28) may result in a portion or even a greater part of ingested lactose reaching the colon for bacterial utilization. That this probably is the case in the adult rat was shown by Koehler and Allen ('34) in experiments showing that lactose had a nutritive value inferior to that of glucose or sucrose on isocaloric diets.

In the diabetic, and to a lesser extent in the obese individual, however, there can be no doubt as to the marked absorption of lactose. At first it seemed possible that through some unknown mechanism lactose might give rise to a hyperglycemia due to mechanical stimulation of the intestines. This possibility was ruled out, however, by the feeding of powdered cellulose. It is difficult to conceive that the enzyme lactase would be present in the diabetic and not in the normal individual. The complete absence of lactosuria or galactosuria in the diabetic after lactose ingestion as compared with its usual presence in the normal individual suggests a difference in the alimentary assimilation, particularly since the diabetic handles intravenously injected lactose much the same as the normal individual.

In considering the possibility of the failure of lactose absorption, it should be remembered that besides the question of the hydrolysis of this disaccharide, the effect of the intestinal rate on the absorption of foodstuffs should be considered. Hosoi, Alvarez and Mann ('28) have shown that lactose passes through the small intestine with great rapidity. Such a rapid rate could easily interfere with hydrolysis and absorption even though lactase were present. Not only could the increased intestinal rate caused by lactose delay its absorption, but it could also interfere with the absorption of other foodstuffs.

SUMMARY

1. The average blood sugar values in sixteen normal young adults after ingestion of 1.5 gm. lactose per kilogram body weight showed no important changes, although considerable individual variations were noted. The secondary hypoglycemic effects were lacking. There was frequently a lactosuria present.

2. The ingestion of 1.5 gm. starch per kilogram caused nearly as much of a peak rise in the blood sugar as obtained after the administration of glucose. The total hyperglycemia after starch is greater than after glucose.

3. The average blood sugar values in sixteen obese individuals showed a slight but definite elevation after lactose ingestion.

4. The blood sugar changes after lactose ingestion in the diabetic were marked and of nearly the same magnitude as after glucose ingestion. In the diabetic cases studied, lactosuria was never noted.

5. The average recovery in the urine of intravenously injected lactose was approximately the same (89.3 to 93.2 per cent) in normal, obese and diabetic subjects.

6. Ingested lactose caused a small fall in the inorganic phosphate content of whole blood.

LITERATURE CITED

BENEDICT, F. G., AND T. M. CARPENTER 1918 Food ingestion and energy transformations with special reference to the stimulating effect of nutrients. Carnegie Inst. of Washington. Publication no. 261.

BERGLUND, H., AND O. FOLIN 1922 Some new observations and interpretations with reference to transportation, retention, and excretion of carbohydrates. J. Biol. Chem., vol. 51, p. 213.

BOURQUELOT AND TROISIER 1889 J. Pharm. Chem., vol. (5), 19, p. 277. Quoted from E. O. Whittier ('25), Lactose Chem. Rev., vol. 2, p. 85.

CAJORI, F. A. 1934 Some characteristics of intestinal lactase. Am. J. Med. Sc., vol. 187, p. 295.

HIGGINS, H. L. 1916 The rapidity with which alcohol and some sugars may serve as nutriment. Am. J. Physiol., vol. 41, p. 258.

HOSOI, K., W. C. ALVAREZ AND F. C. MANN 1928 Intestinal absorption. Arch. Int. Med., vol. 41, p. 112.

KJER, K. 1924 Investigations into the relation of blood sugar to starch in normal persons. Acta Med. Scand., vol. 61, p. 159.

KOEHLER, A. E., AND S. E. ALLEN 1934 The nutritive value of lactose. J. Nutrition, vol. 8, p. 377.

MCCULLAGH, E. P., AND D. R. MCCULLAGH 1932 Carbohydrate in the treatment of post-operative tetany with special reference to lactose. J. Lab. Clin. Med., vol. 17, p. 754.

RELATION OF CASTRATION TO VITAMIN A-DEFICIENCY IN THE RAT [1]

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TWO FIGURES

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It has been demonstrated that an adequate supply of vitamin A is essential for the maintenance and structural integrity of the germinal epithelium of the testis of the rat (Mason, '30, '33; Evans, '32). On the basis of these observations, it seemed of interest to determine whether the amount of A actually utilized by the testis was of sufficient magnitude to produce a significant delay in the appearance of the deficiency symptoms in rats completely castrated before being placed on vitamin A-deficient diets, as compared with non-castrate rats.

In a series of experiments involving seventy-eight rats and conducted during the fall and winter of 1929–1930, observations made incidental to other studies for which the animals were being prepared suggested that xerophthalmia was retarded in its appearance in castrated male rats, when compared with their non-castrate brothers (Wolfe and Mason, '30). The eye changes appeared in the castrates at about the peak of the growth curve; whereas in non-castrate rats these changes appeared some time before the growth peak was attained. However, similar observations were subsequently noted in a series of ten castrate female rats and ten non-castrate littermate sisters. Since it did not seem possible that

[1] This investigation was aided by grants from the Commonwealth Fund and from the Division of Medical Sciences of the Rockefeller Foundation.

the ovary utilized sufficient vitamin A to produce any significant difference in the manifestation of the deficiency, it was thought that an altered metabolism following castration might afford a more logical explanation.

To test this hypothesis, the respiratory quotient and basal metabolic rate were determined on a series of rats including controls, castrated controls, A-deficient non-castrates and

TABLE 1

Respiratory quotient and metabolic rate of A-deficient and control rats

RAT	TREATMENT	RESPIRATORY QUOTIENT		BASAL METABOLIC RATE		
		First determination	Second determination	First determination	Second determination	Average
41-15[1] ♂	-A	.74		772		839
-16 ♂	-A	.72		906		
41-17 ♂	-A castrate	.75		1095		989
-18 ♂	-A castrate	.73		883		
41-19 ♂	Control	.74		1001		1038
-20 ♂	Control	.74		1075		
41-21 ♂	Control castrate	.75		888		934
-22 ♂	Control castrate	.75		980		
40-50 ♂	-A castrate	.76	.76	898	1103	1001
-61 ♀	-A castrate	.77	.74	1076	975	1026
-68 ♀	-A castrate	.75	.75	831	930	881
-70 ♀	-A castrate	.73	.74	870	880	875

[1] Rats 41-15 to 41-22 were all litter-mate brothers.

A-deficient castrates.[2] By careful regulation of the diet, a constant and moderately severe degree of xerophthalmia was maintained in the A-deficient rats during a period of from 60 to 100 days prior to the metabolic studies. The data obtained failed to demonstrate any metabolic differences sufficiently significant to explain the delayed appearance of xerophthalmia in castrated rats (table 1).

[2] Acknowledgment is made to Dr. L. G. Wesson, of the Department of Pharmacology, for his kindness and cooperation in making these metabolic studies. The method used was essentially that previously described by Wesson ('27).

In our earlier studies (Wolfe and Mason, '30) examination of the eyes was made bi-weekly, and not always by the same individual; which made it difficult to interpret the exact time at which xerophthalmia appeared. Furthermore, certain of the animals exhibited a mild chronic type of deficiency with temporary appearance of early signs of xerophthalmia at intervals, before more advanced stages became apparent. The inadequacies in the data warranted repetition of these experiments with more careful observations on the eye changes. During the following year, careful daily examination of the eyes of a series of sixteen A-deficient male rats, half of which were castrated before being placed on the experimental diet, gave no significant difference in the time of appearance of the eye changes in the two groups. It then seemed advisable to restudy the whole problem using female rats, in order to apply another criterion of A-depletion, namely, the abnormal vaginal cornification first described by Evans and Bishop ('22) and now generally accepted as a reliable indicator of vitamin A-deficiency. The results of these newer studies are outlined below.

The 102 rats selected for this study (series A and B, table 2) averaged approximately 40 gm. body weight (minimum 37 —maximum 46) and varied in age from 19 to 26 days when put on experiment. Both albino and pied strains were used, with no observable difference in the results secured. Litter-groups were equally divided between castrates and non-castrates, except where odd numbers were present. In the latter instance, the odd member was placed in the non-castrate group. Castration was performed just prior to the beginning of experimental feeding. The diets used were essentially the same as those used in earlier studies mentioned above. Their composition was as follows:

Diet 66	*Per cent*	*Diet 67*	*Per cent*
Casein (purified)	18	Casein (unextracted)	18
Starch	58	Starch	58
Lard	20	Lard	20
Salt mixture	4	Salt mixture	4

TABLE 2

Showing the average time of appearance of continuous vaginal cornification and xerophthalmia in A-deficient castrate and non-castrate rats

SERIES		NUMBER OF RATS	WHEN STARTED		PERIOD ON EXPERIMENT BEFORE CONTINUOUS VAGINAL CORNIFICATION			PERIOD ON EXPERIMENT BEFORE DEFINITE AND CONTINUOUS XEROPHTHALMIA APPEARED			INTERVAL
			Average weight, grams	Average age, days	Days	Minimum	Maximum	Days	Minimum	Maximum	
Series A (diet 65)	Non-castrates ♀	26	40.4	23.5	21.2	15	26	33.7 (22 rats)	25	44	10.2
	Castrates ♀	21	41.7	22.5	22.5	18	27	31.6 (20 rats)	25	42	9.1
	Non-castrates ♂	7	41.3	24.0	....	..	..	32.0	24	41	....
	Castrates ♂	6	42.3	24.0	....	..	..	32.0	26	40	....
Series B (diet 67)	Non-castrates ♀	24	41.5	21.0	21.7	17	27	43.0 (22 rats)	30	48	21.3
	Castrates ♀	18	43.1	21.0	23.1	17	27	42.5 (17 rats)	30	50	19.4
Series C (diet 67)	Non-castrates ♀	34	40.0 min. 37 max. 43	23.0	22.5	19	26	34.8	25	45	12.3
Series D (diet 67)	Non-castrates ♀	48	65.0 min. 50 max. 78	28.0	40.4	35	55	Used for other experiments after the seventieth day. At that time xerophthalmia had not appeared in any of the group			

In addition to the above diet, each rat received, by separate daily administration, 0.5 to 1 gm. of dried yeast and 0.005 gm. of irradiated ergosterol.

Daily vaginal smears (dried and stained in hematoxylin and eosin) were begun on the fourteenth day of experiment, the vaginal membrane being ruptured if not already open. The eyes were examined daily with the aid of a strong hand lens and good illumination. Body weights were recorded on alternate days. The rats were maintained throughout the experiment in individual, false bottom, wire cages in an airy well-lighted room, under uniform experimental conditions. All observations on the vaginal smears and eye-changes were made by the same individual.

From the results of series A (table 2), represented graphically in figure 1, it is apparent that continuous vaginal cornification not only appeared much earlier, but also showed much less variation in time of appearance than did the eye changes. Furthermore, the average time of appearance of both symptoms of A-deficiency was essentially the same for the castrate and non-castrate groups, the small differences being no greater than one might expect in determining the midpoint of the transition period between normal and abnormal vaginal or eye conditions. When careful comparisons were made between castrates and non-castrates from the same litter group, no significant differences in the time of onset of the deficiency symptoms were observed.

Similar results were obtained in the rats of series B, in which unextracted casein was used.[3] It is of interest to note that, although continuous vaginal cornification appeared at approximately the same time in both series, the small traces of A in the unextracted casein of series B significantly re-

[3] We have repeatedly demonstrated that the casein used in our studies during the past 6 years, a finely pulverized product obtained from A. Adler & Sons, Philadelphia, contained only small traces of vitamin A. Unless a very rapid and severe deficiency was desired in the experimental animals, the difficulty and expense of alcoholic extraction has not seemed warranted. In this connection, we would like to correct a statement made in a previous report (Mason, '33, p. 172, footnote 10), stating that the fat content of the above casein was 0.04 per cent. This was intended to refer to the extracted, and not the commercial, product.

tarded the appearance of xerophthalmia and, in some instances, either no clear-cut eye symptoms appeared or else mild symptoms were observed to alternate, every few days, with complete absence of xerophthalmia. In all the rats of series A, and in the majority of those of series B, once the eye symptoms appeared they became progressively worse and reached their maximum severity within 2 weeks or less.

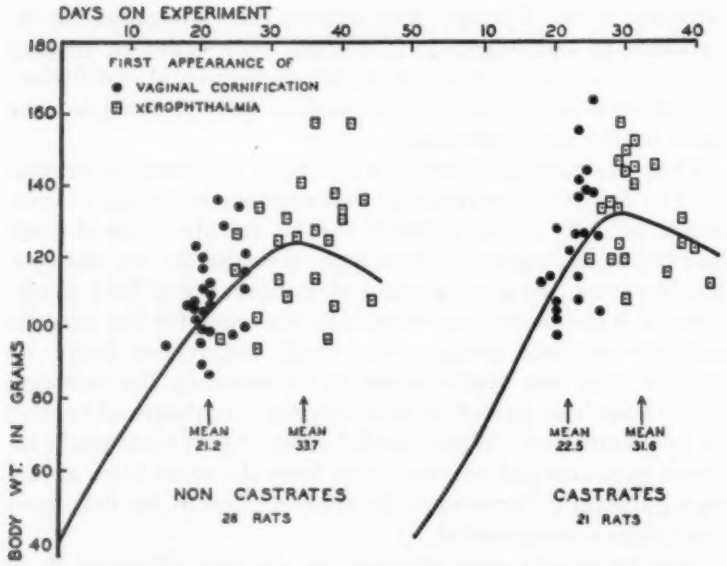

Fig. 1 Showing the average growth curve and the time of appearance of continuous vaginal cornification and of xerophthalmia in the individual A-deficient female rats of series A, table 2.

Only seven non-castrate and six castrate males were used (series A). Xerophthalmia appeared at approximately the same time in all instances. Since this was in accord with previous findings on a group of sixteen male rats, half of which were castrated, and confirmed similar observations on the female rats of series A and B, additional studies on males did not seem necessary. On the basis of the data presented

above, it can be concluded that complete castration of the male or female rat had no demonstrable effect upon the development of vitamin A-deficiency.

The combined data from 126 rats, 114 of which are included in series A, B and C (table 2), presented graphically in figure 2, convincingly demonstrate the superiority of the vaginal smear test as a criterion of early vitamin A-deficiency. These rats weighed approximately 40 gm. when placed on the ex-

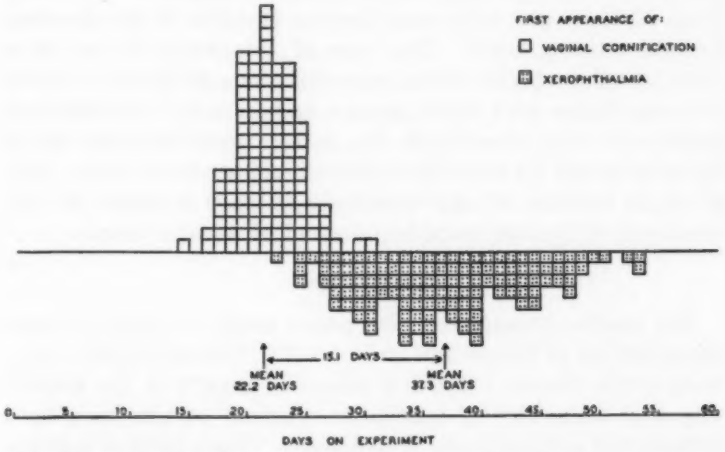

Fig. 2 Showing the distribution frequency of the time of appearance of continuous vaginal cornification and of xerophthalmia in 126 A-deficient rats (castrate and non-castrate) including 114 of those of series A, B and C (table 2).

perimental diet. The average time required to induce continuous cornification was 22.2 days, with a maximum variation of 17 days; whereas the average time before xerophthalmia appeared was 37.3 days, with a maximum variation of 32 days. In other words, rats placed on a diet relatively low in vitamin A after attaining body weights of 40 gm. developed continuous vaginal cornification approximately 2 weeks prior to the appearance of definite xerophthalmia.

In general, the earlier the onset of vaginal cornification and of xerophthalmia, the smaller the time interval between the

appearance of these two criteria. We have frequently noted an almost simultaneous appearance of these criteria before the end of the suckling period in young rats whose mothers were maintained on low levels of vitamin A-intake. When rats from the stock colony, with a moderate storage of vitamin A and weighing approximately 40 gm., were placed on the experimental diets (series A, B and C, table 2) the average of the interval between the appearance of the two symptoms varied from 9.1 to 21.3 days. When young rats weighing from 50 to 75 gm. were used (series D, table 2) the interval became much greater. The rats of this latter series were used for other studies before xerophthalmia developed. From our experience with other groups, in which the experimental conditions were essentially the same, xerophthalmia would have appeared between the eightieth and ninetieth days. This gives an interval of approximately 45 days between the appearance of vaginal cornification and of xerophthalmia.

DISCUSSION

The studies presented in this paper amply confirm previous observations of Evans and Bishop ('22), Baumann and Steenbock ('32), Aberle ('33) and others in regard to the sensitivity and reliability of continuous vaginal cornification as a criterion of early vitamin A-deficiency. They further indicate that castrate rats offer no additional advantage over non-castrate rats, in ascertaining the onset of the abnormal changes. It should be stated, however, that in the present studies, involving comparison of castrate and non-castrate litter-mate sisters (series A and B, table 2), abnormal cornification appeared before the first oestrus in 78 per cent of the non-castrate rats and before the approach of the third oestrous period in the remainder. There is no evidence that a greater degree of oestral activity in the vaginal epithelium prior to the onset of A-deficiency changes would have altered the time of appearance of the latter, resulting in a greater difference between castrates and controls in this respect.

On the other hand, the castrate rat has proved much less satisfactory than the non-castrate in following the repair of the vaginal cornification after vitamin A-therapy, as will be discussed more fully in a later report dealing with the histological details of the vaginal smear after A-deficiency (Mason and Ellison, '35). The slightly superior growth of the castrates in the present series (fig. 1) as well as in the earlier studies mentioned above, was first thought to be due to the intentional selection of the larger animals of each litter group for castration with the idea of thereby offsetting a possible early retardation due to the operation. But the latter seemed to have no significant effect on the subsequent growth of the animals. This may be better explained by the recent observations of Beebe ('32) that "an increase in weight and in food consumption occurs in rats following ovariectomy, even in the face of vitamin A-deficient diets. The beneficial effect of ovariectomy is especially pronounced when performed early in life."

CONCLUSION

1. Complete castration caused no significant difference in the time of appearance of continuous vaginal cornification or of xerophthalmia in A-deficient rats of either sex.

2. Continuous vaginal cornification was subject to much less individual variation, and could be detected approximately 2 weeks earlier, than xerophthalmia, in rats placed on the diet at body weights of 40 gm.

3. Castrate rats were not superior to non-castrates for determining the beginning of vaginal cornification.

LITERATURE CITED

ABERLE, S. B. D. 1933 Continual cornified vaginal cells as an index of avitaminosis-A in rats. J. Nutrition, vol. 6, pp. 1–10.

BAUMANN, C. A., AND H. STEENBOCK 1932 The vaginal smear method of determining vitamin A. Science, vol. 76, pp. 417–420.

BEEBE, I. J. 1932 The effect of bilateral ovariectomy upon rats on a vitamin A-deficient diet. Yale J. Biol. and Med., vol. 5, p. 196.

EVANS, H. M. 1932 Testicular degeneration due to inadequate vitamin A in cases where E is adequate. Am. J. Physiol., vol. 99, pp. 477–486.

EVANS, H. M., AND K. S. BISHOP 1922 On an invariable and characteristic disturbance of reproductive function in animals reared on a diet poor in fat soluble vitamine A. Anat. Rec., vol. 23, p. 17.

MASON, K. E. 1930 The specificity of vitamin E for the testis. I. Relation between vitamins A and E. J. Exp. Zoöl., vol. 55, pp. 101–122.

———— 1933 Differences in testis injury and repair after vitamin A-deficiency, vitamin E-deficiency, and inanition. Am. J. Anat., vol. 52, pp. 153–239.

MASON, K. E., AND E. T. ELLISON 1935 Changes in the vaginal epithelium of the rat after vitamin A-deficiency. J. Nutrition, vol. 9, pp. 735–755.

WESSON, L. G. 1927 An apparatus and method for the determination of the respiratory quotient of small animals. J. Biol. Chem., vol. 73, pp. 499–506.

WOLFE, J. M., AND K. E. MASON 1930 The effect of castration on the development of vitamin A-deficiency in the albino rat. Anat. Rec., vol. 45, p. 282.

CHANGES IN THE VAGINAL EPITHELIUM OF THE RAT AFTER VITAMIN A-DEFICIENCY [1]

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TWO PLATES (TWENTY FIGURES)

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Excessive cornification of the vaginal epithelium in the rat is a characteristic and early manifestation of vitamin A-deficiency. The first observation of this phenomenon by Evans and Bishop ('22) has been repeatedly confirmed by many investigators. By the majority of these, the continued presence of cornified cells in the vaginal smear has been regarded as a reliable index of early depletion of vitamin A (Macy, Outhouse, Long and Graham, '27; Hohlweg and Dohrn, '30; Baumann and Steenbock, '32; Aberle, '33; Mason and Ellison, '34; Mason and Wolfe, '35). Others have considered this phenomenon unsatisfactory for diagnostic purposes (Coward, '29; Coward, Morgan and Dyer, '30; Thatcher and Sure, '32). This whole problem is more completely reviewed in a recent report by Baumann and Steenbock ('32), to which the reader is referred.

Normally, cornified cells appear in the vaginal smear of the rat only during about one-third of the total oestrous cycle (oestrus and metoestrus). Their constant presence in A-deficient rats does not indicate a continuous state of oestrus, for ovulation and corpora lutea formation occur at regular or irregular intervals, as indicated by successful matings

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with normal males (Evans and Bishop, '22; Evans, '28). Except for the latter studies, no attempt has been made to demonstrate rhythmic oestrous changes in A-deficient animals.

In an extensive analysis of the effect of vitamin A-deficiency on the female reproductive system of the rat, representing a continuation of similar studies on the male rat (Mason, '33), we have had occasion to study more than 52,000 vaginal smears obtained from more than 500 A-deficient and normal rats during the past 4 years. In preliminary studies (Mason and Wolfe, '35), it was demonstrated that abnormal cornification of the vaginal smears usually appears much earlier than do the other criteria of vitamin A-deficiency, and affords a very sensitive and reliable index of sub-xerophthalmic levels of the deficiency disease.

In the present report, the essential alterations observed in the vaginal smears during the progressive development of vitamin A-deficiency, and during repair of the latter, are described; and an interpretation of the manner in which lack of A influences the vaginal epithelium is offered.

METHOD

In making routine dry-stained vaginal smears on a large number of rats, the following method has proved most satisfactory. The numbers of the rats to be smeared are marked on glass slides, ruled transversely into twelve to fifteen rectangular areas, by means of a diamond pencil. The vaginal scrapings, obtained by means of a narrow aluminum spatula, are smeared as evenly as possible on the space opposite the proper number. Between each smearing, the spatula is thoroughly cleaned. Care should also be exercised in procuring the smears such that cornified cells from the external surface of the vaginal orifice are not included.

The smears are stained with Ehrlich's or Delafield's hematoxylin for ½ to several hours, washed gently with water, deeply blued with ammonia vapor, washed, faintly counterstained in eosin, washed and dried. It is important to secure an intensely blue stain sufficiently contrasted with, but not

overshadowed by, the eosin counterstain. Microscopic examination of the entire smear is necessary, because of the variation in the proportion of different cell types in different parts of the smear. From this composite picture, the general characteristics of the smear can be estimated.

The diets used were composed of casein, starch, lard or Crisco, salt mixture, dried brewer's yeast, and irradiated ergosterol, and were of essentially the same composition as those used in earlier studies (Mason, '33; Mason and Wolfe, '35). Vitamin E was supplied by separate administration of wheat germ oil with the yeast supplement. When introduction of traces of vitamin A was not desired, the wheat germ oil was aerated at 100°C. for a period of 4 hours.

A. ABNORMAL VAGINAL CORNIFICATION OF A-DEFICIENCY

When the rat is rapidly depleted of vitamin A the interval between the first appearance of abnormal vaginal smears and the onset of complete and continuous vaginal cornification varies from a few days to a week. The description of the successive stages of cornification, as outlined in this paper, is based upon the vaginal smears of rats in which vitamin A-deficiency developed less acutely, in order to bring out more clearly the progressive changes which occur.

Early cornification. The first appearance of abnormal cornification in the dioestrous type of smear (fig. 1) was indicated by variable degrees of swelling, vacuolation and keratinization of the nucleated epithelial cells (fig. 2), and by a thin watery appearance of the mucus when present in such smears (fig. 3). Usually cornification was complete in only a relatively small number of the cells. Since such smears frequently resembled those of late metoestrus (fig. 8), continued cornification of the smear during several successive days was necessary for the differentiation of these two conditions. These early changes, and the increasing degrees of cornification which follow, were most clearly portrayed during prolonged periods of dioestrus; such as occur after castration and during pregnancy, pseudopregnancy and lactation. When

the cyclic changes of oestrus occurred, the progressive cornification of the smears was interrupted by the normal processes of epithelial cornification, dehiscence and regeneration resulting from hormonal influences. The modification of the latter during the early stages of A-deficiency cornification will now be considered.

The normal oestrous cycle of the rat usually varies from 4 to 6 days in length, the general average approximating 5 days (Long and Evans, '22). Successive daily smears usually show the stages of prooestrus (figs. 5 and 6), oestrus (fig. 7), and metoestrus (fig. 8), followed by several days during which the smear of dioestrus (fig. 1) appears. Only infrequently does the smear of oestrus appear on 2 successive days.

As the vaginal epithelium became altered by lack of vitamin A, striking changes occurred in the prooestrous smear. Frequently, these changes were detectable at the transition period between late dioestrus and early prooestrus, in the pale swollen appearance of many of the large nucleated epithelial cells and in the watery appearance of the mucus, when present (fig. 4). At other times, the large basophilic nucleated epithelial cells so characteristic of normal prooestrus (fig. 5) appeared greatly swollen, and exhibited considerable cornification. The nuclei were very pycnotic and the cell outlines were usually rather fuzzy and indistinct (fig. 9). This type of smear somewhat resembled that of the transition period between normal prooestrus and normal oestrus (fig. 6), but was distinguishable from the latter by the larger size and more cornified nature of the epithelial cells. As the deficiency progressed, the atypical prooestrous smear soon became difficult to distinguish from that of oestrus. Its early obliteration afforded a striking and trustworthy criterion of early cornification of A-deficiency, and was usually followed by an apparent prolongation of oestrus over 2 successive days.

Moderate cornification. Following the appearance of the smears of atypical prooestrus, and of prolonged oestrus, the smears of dioestrus became more and more indistinguishable from those of metoestrus. They were characterized by an

increase in the number of completely cornified nucleated epithelial cells and, particularly, by the predominance of large and small nucleated epithelial cells exhibiting intermediate stages of cornification (fig. 10). The acidophilic staining reaction of the majority of the epithelial cells gave a somewhat pinkish tinge to the entire smear. Not more than 10 to 15 per cent of the nucleated epithelial cells remained unaltered. Leucocytes were variable in number, representing from 20 to 40 per cent of the cells present. Mucus never appeared in the smear after early cornification became manifest. The material removed each day, by gentle insertion of the spatula, was cheesy in consistency and became more and more abundant as the cornification progressed.

Advanced cornification. As the cornified smear of proestrus merged with those resembling prolonged oestrus, the oestrous cycle was represented by approximately three successive daily smears in which cornification was complete, or practically so, alternating with approximately two successive daily smears in which a relatively small number of leucocytes and partially cornified cells made their appearance among the completely cornified cells. The latter represented the increasing cornification of the dioestrous type of smear (figs. 11 and 12), in which the marked reduction in non-cornified nucleated epithelial cells was much more characteristic than was the relative number of completely cornified cells and leucocytes present.

Extreme cornification. As the deficiency progressed, the smear of dioestrus contained a still greater abundance of large flaky cornified cells but very few leucocytes and partially cornified cells (fig. 13). Eventually, the dioestrous smear contained only cornified cells (fig. 14) and closely resembled that of oestrus (fig. 7). This latter condition of complete and continuous vaginal cornification usually developed before definite xerophthalmia and growth retardation had become apparent. The cyclic changes of oestrus then became obliterated by the excessive cornification and could be followed only by the supravital method of examination of the vaginal smears (Mason and Ellison, '35).

Vaginal cornification during pregnancy. During middle and late pregnancy, in the normal rat, large nucleated cells undergoing a mucoid type of transformation are frequently observed in the vaginal smear (fig. 15). They represent the cells forming the thick mucified epithelial lining of the vagina typical of late pregnancy (fig. 16). When a mild deficiency of vitamin A existed, these cells could be observed undergoing different degrees of cornification (fig. 17). When A-depletion was more severe, this mucoid transformation was entirely suppressed and the vaginal epithelium was thickened and cornified throughout pregnancy (fig. 19).

Slightly higher levels of vitamin A appeared to be necessary for maintenance of the mucified state of the vagina during late pregnancy than for maintenance of normal vaginal smears in non-pregnant rats. It has not been possible to determine whether this was due to a greater need for A on the part of the mucified epithelium, or to a greater drainage of the maternal stores of A during late pregnancy.

B. RHYTHMICITY OF OESTRUS

The periods of complete but temporary cornification of the vaginal smears, mentioned above, did not represent a state of continuous 'heat.' The smear of the first day of such periods constituted the cornified smear of prooestrus, and pycnotic eosinophilic nuclei were frequently discernible in the cornified cells at this stage. During the mid part of these periods the cornified cells were rather indistinct in outline and tended to clump. During the last day, the cells were more discrete and more evenly distributed in the smear, bearing a closer resemblance to those of normal oestrus (fig. 7). Furthermore, as demonstrated by more than 100 mating tests with normal males, copulation occurred only during the last day of these periods. Hence, only the latter represented the period of true oestrus, or 'heat.'

With this period of true oestrus as a guide, oestrous cycles of normal duration (table 1) could be followed as long as periods of complete but temporary cornification continued to

alternate with 1 or more days of partial cornification (figs. 10 to 13). Only during more advanced stages of A-deficiency, associated with definite growth retardation, did the oestrous cycle become irregular and lengthened (Mason and Ellison, '35).

TABLE 1

Showing the regularity of oestrus prior to and following abnormal cornification of A-deficiency

NUMBER OF RATS IN LITTER	INITIAL BODY WEIGHT, AVERAGE	BODY WEIGHT AT BEGINNING OF VAGINAL CORNIFICATION, AVERAGE	DAYS ON EXPERIMENT BEFORE VAGINAL CORNIFICATION APPEARED, AVERAGE	BEFORE VAGINAL CORNIFICATION		AFTER VAGINAL CORNIFICATION	
				Number of oestrous cycles	Average length of cycles	Number of oestrous cycles	Average length of cycles
	gm.	*gm.*			*days*		*days*
5	74	188	43	26	5.22	29	5.0
4	71	169	44.5	20	5.25	24	5.0
5	70	168	47	29	5.1	8	4.75
4	68	189	51	26	5.0	29	5.0
4	66	182	56	33	4.67	22	4.6
3	64	173	41	10	5.0	24	5.58
2	62	176	53.5	13	4.23	12	4.33
3	60	180	45	10	4.8	13	5.23
3	57	163	37	11	6.3	25	5.7
4	54	138	42	12	5.4	4	5.0
Total 37				190		190	
Average[1]					5.04		5.08

[1] Based on values for individual rats.

C. REPAIR OF VAGINAL CORNIFICATION BY A-THERAPY

1. Non-castrates. The sequence of events seen in successive daily smears following A-therapy was essentially the reverse of that occurring during the onset of cornification as described above, and could be best followed in rats receiving from one-tenth to one-fifth drop of cod liver oil, daily. The latter represents the critical level of therapy for maintenance of normal smears in A-deficient rats (table 2).

The time interval required to effect repair of the vaginal epithelium (as judged by the disappearance of cornified cells of A-deficiency from the vaginal smears) varied from 1 to 13 days (table 2), depending upon the amount of A supplied.

With the higher levels of therapy, and the shorter intervals of repair, the onset of oestrus within a few days of the beginning of therapy led to a considerable range of error in determining the exact time at which repair was completed.

Mucus, which was characteristically absent from the vaginal smear after the first appearance of cornification, reappeared at about the same time that normality of the smears was established. For a week or so following the disappearance of abnormal cornification in the vaginal smears, considerable

TABLE 2

Showing the repair of A-deficiency cornification of the vaginal epithelium following different levels of vitamin A-therapy

NUMBER OF TESTS	AMOUNT OF COD LIVER OIL ADMINISTERED, DROPS	DURATION OF CORNIFICATION BEFORE THERAPY, DAYS			DAYS BEFORE VAGINAL SMEARS FREE OF CORNIFICATION			DURATION OF EFFECT
		Aver.	Min.	Max.	Aver.	Min.	Max.	
4	1 dr. single dose	25	20	30	4	3	5	1 to 6 days
39	2 dr. single dose	23	5	58	4	2	7	7 to 14 days
5	4 dr. single dose	46	40	53	2	2	3	15 to 20 days
14	1/10 dr. daily	19	10	28	11	9	13	Subminimal in 9 rats
15	1/5 dr. daily	18	8	24	11	8	12	Subminimal in 4 rats
12	1/2 dr. daily	20	15	42	5	3	8	.....
6	1 dr. daily	19	16	26	4	3	6	.....
30	6 dr. daily	16	..	..	2	1	3	.....
6	Stock diet, daily	28	14	36	10	8	12	.....

vacuolation of the nucleated epithelial cells was observed. They appeared to represent deeper cells of the epithelium which had not fully recovered from the effects of the deficiency state. Such cells were also demonstrable, at times, in the smears of normal rats. The reappearance of mucus, and the disappearance of vacuolation in the nucleated epithelial cells, did not prove to be satisfactory criteria for determining the end point of repair.

The rapidity and completeness of repair following A-therapy was well demonstrated in a group of twenty rats given

6 drops of cod liver oil, daily, beginning on the twelfth day of pregnancy; at which time the vaginal smears were extremely cornified. Within approximately 5 days cornified cells were entirely absent from the smears and large mucoid nucleated epithelial cells were present in abundance. The rapid response of the vaginal and urethral epithelia to small amounts of vitamin A is shown in figure 20.

In the tests presented in table 2, the duration of vaginal cornification prior to A-therapy varied from 5 to 58 days. Provided advanced or severe cornification (figs. 11 to 14) persisted for approximately 1 week, the previous duration of cornification had no significant effect upon the repair response of the vaginal epithelium. Other data, presented in table 2, indicate that the usefulness of the vaginal smear test as a method of bio-assay lies in the time interval between the repair of abnormal cornification and reappearance of the latter, following the administration of single doses of vitamin A-containing substances, rather than in the time interval required to effect repair.

2. Castrates. Forty castrate rats, in which vaginal cornification had existed for 2 to 4 weeks, were used for A-therapy tests. The dosages of cod liver oil supplied were the same as given in other tests where non-castrate rats were used (table 2), but the results were quite different. There was a marked reduction in the cornified cells of the smears, and normal nucleated cells and leucocytes appeared in large numbers. But small numbers of cornified cells usually continued to appear either constantly or at irregular intervals throughout the duration of the test, which varied from 3 to 12 weeks. There was usually no clear cut end point of repair such as observed in the non-castrate rat.

Mucus made its appearance in the smear soon after therapy was begun, and persisted throughout the period of therapy. Histologically, the vaginae were typical of the castrate condition, except for the presence of small numbers of cornified cells in the superficial layers of the thin epithelium (two- to four-cell layers), and for the presence of unusually large

numbers of leucocytes forming a layer several cells deep just internal to the vaginal epithelium.

DISCUSSION

Many excellent studies of the past 10 years have offered convincing evidence that the primary effect of vitamin A-deficiency is an alteration in the epithelial cells throughout the body. The present paper adds further evidence in support of this concept, and demonstrates the reliability and usefulness of the vaginal smear test as a criterion of mild sub-xerophthalmic levels of vitamin A-deficiency.

According to the observations of Wolbach and Howe ('25, '33), Seifried ('30), and others, the fundamental effect of lack of vitamin A appears to involve an atrophy of the epithelium accompanied by, or followed by, a reparative proliferation of the basal cells. The latter, having lost their type specificity, produce a stratified keratinizing epithelium regardless of the type previously existing in that location. In recovery, each epithelium returns to its normal type (Wolbach and Howe, '33). The extent and the nature of disturbances of secretory function in epithelial cells has received very little attention.

In the vaginal epithelium, which is normally stratified and which periodically cornifies, no primary atrophy was observed after A-deficiency. But a marked increase in the proliferative activities of the epithelium was striking, as demonstrated by the increased abundance of cellular material obtained in the daily process of smearing as the deficiency progressed, and by the greatly increased thickness of the epithelium, especially in castrate rats. These observations are in accord with the increase in mitotic activity noted in the vaginal epithelium of A-deficient castrate rats by Aberle ('33 a), and with the interpretation of the metaplasia in other epithelia as observed by Wolbach and Howe ('25, '33) and others.

More striking than the increased stratification and metaplasia of the vaginal epithelium was the increased cornification of the epithelial cells, especially when cyclic changes of oestrus were occurring. In the normal rat, after the restitu-

tion of the vaginal epithelium at the approach of prooestrus, the large nucleated epithelial cells forming the internal lining of the epithelium are separated from the basal layer (stratum germinativum) by a layer of cells in which cornification occurs (stratum corneum); these two more superficial layers forming the cells of the prooestrous and oestrous smears, respectively. The obliteration of the prooestrous smear, and the increased duration of the oestrous type of smear, during the earlier phases of A-deficiency, seemed attributable to premature cornification of the cells normally forming the superficial nucleated layer and to an accentuation of the cornification normally occurring in the stratum corneum. The increased cornification of the dioestrous smear appeared to be due to less complete removal of this more cornified superficial layer of the epithelium during the degenerative changes of metoestrus, and to abnormal cornification of the deeper layers of cells, culminating in complete and continuous cornification of the smear.

That severe and prolonged cornification of the vaginal epithelium produced no permanent injury was indicated by the rapidity of repair following A-therapy, as noted also by other investigators (Evans, '28; Baumann and Steenbock, '32; Aberle, '33). The more rapid repair in this epithelium, as compared with that observed by Wolbach and Howe ('33) in other types of epithelia, appears to be due to two factors. The vaginal epithelium requires no reorganization after the influences inducing excessive cornification are removed. Furthermore, the cyclic dehiscence and regeneration of the outer layers of the epithelium associated with the oestrous cycle appeared to greatly facilitate the repair process.

The incompleteness of removal of cornified cells from the vaginal smears of A-deficient castrate rats after vitamin A-therapy has received no satisfactory explanation. It seems possible that the absence of the periodic degeneration and reorganization of oestrus, and decreased mitotic activity of the cells of the basal layer of the epithelium, associated with a more deep-seated injury induced by the previous state of

A-deficiency in the castrate epithelium, where cyclic changes of oestrus are absent, are important factors. Contrary to the belief of Hohlweg and Dohrn ('30), we feel that the castrate rat is unsuitable for tests involving the repair of vaginal cornification by A-therapy. However, in determining the time of appearance of vaginal cornification in A-deficiency, castrates prove as useful as non-castrates, but offer no advantage over the latter (Mason and Wolfe, '35).

The inability of the vaginal epithelium of A-deficient rats to produce mucified epithelial cells normally characteristic of pregnancy suggests a definite alteration in the physiologic state of the cell, rather than a deficiency of hormonal stimuli acting upon the cell. This is supported by our inability to demonstrate any significant alteration in the endocrine functions of the ovary or of the hypophysis (Mason and Wolfe, unpublished studies), and by the observations of Aberle ('33 a) that the vaginal epithelium of the A-deficient rat is quite incapable of responding to excessive administration of a placental extract which regularly induces marked mucification of this epithelium in the normal rat. It appears that, in epithelial cells deprived of adequate vitamin A, the production of glyco-proteins (mucin) is interfered with and albuminoids (keratin) appear in excess. This would suggest that vitamin A may play an important role in protein metabolism or synthesis within the epithelial cell. In the present studies, it has not been possible to determine whether the keratinizing process of A-deficiency differs, in any respects, from that normally occurring at the period of oestrus in normal rats under the influence of hormonal factors.

The observations presented in this report, and in a separate paper (Mason and Wolfe, '35), clearly demonstrate the reliability of vaginal cornification as an index of early stages of vitamin A-deficiency. These findings are in disagreement with those of Parkes and Drummond ('26), Coward ('29), Coward, Morgan and Dyer ('30) and Thatcher and Sure ('32), as discussed more fully elsewhere (Mason and Ellison, '35).

As a method for the biological assay of vitamin A, the vaginal smear test seems best applicable to the measurement of the interval elapsing between the repair of, and the return of, vaginal cornification following administration of single doses of vitamin A-containing substances. Unlike the other criteria of A-deficiency (xerophthalmia and growth plateau), vaginal cornification is not significantly influenced by other factors (differences in inherent growth capacity of the test animal, general debility, extraneous causes of ocular irritation and infection) which may alter the reparative response when measured by other methods of assay. The lesser degree of A-depletion required in the test animal, and the utilization of the same animal for repeated tests at intervals of a few weeks, offer additional advantages.

As far as we can ascertain, no attempt has yet been made to determine whether lack of vitamin A produces increased cornification of the vagina in the human. The evidence at hand would suggest that vitamin A is essential for the maintenance of a normal healthy state of this epithelium. Of interest in this latter connection are recent observations of Dr. J. W. Simpson, of the Department of Obstetrics and Gynecology of this school (personal communication), dealing with the efficacy of a high intake of vitamin A in the treatment of senile vaginitis, especially in patients in whom the dietary history suggested a low intake of this factor. Not only were the clinical and macroscopical manifestations much more rapidly improved than by the more usual methods of treatment, but small portions of the vaginal epithelium removed by biopsy, at intervals during treatment, showed marked histologic evidences of repair.

The usefulness of the vaginal smear test in detecting early stages of vitamin A-deficiency in the rat, and in permitting study of the injury induced by mild levels of A-deficiency undetectable by other pathognomonic signs, is demonstrated in a separate study (Mason, '35).

SUMMARY AND CONCLUSIONS

1. The changes occurring in the dry stained vaginal smears of castrate, non-castrate, and pregnant rats during the development of vitamin A-deficiency, and during repair of the deficiency state, are described. These observations are based upon the study of more than 52,000 vaginal smears.

2. Lack of A results in an early cornification of the nucleated epithelial cells typical of the prooestrous smear, and in an accentuation of the process of cornification normally occurring at oestrus, resulting in the appearance of completely cornified smears during several successive days. The last day of such periods represents the stage of true oestrus, or 'heat.'

3. There also occurs a progressive cornification and an increased proliferation of the nucleated epithelial cells of the dioestrous type of vaginal smears, associated with a corresponding reduction in the relative number of leucocytes, culminating in continuous and complete cornification of the smears before xerophthalmia and growth retardation become apparent.

4. As a result, the cyclic changes of oestrus are represented by alternating periods of partial (metoestrus and dioestrus) and complete cornification (prooestrus and oestrus) of the vaginal smears. Until the two types of smear merge into a state of complete and continuous cornification, oestrous cycles of normal duration can be followed by the dry stained smears.

5. Mucus disappears from the smear at the onset of cornification and reappears during the early stages of repair.

6. Except in castrate rats, daily administration of one-tenth to one-fifth drop of cod liver oil usually effects repair of severe vaginal cornification during the second week of therapy, and represents the approximate minimal level of therapy for maintenance of normal vaginal smears in A-deficient rats. With larger doses of vitamin A, cornified cells disappear from the smear within 1 to 2 days.

7. Small numbers of cornified cells continue to appear, in the vaginal smears of castrate rats, even after long periods of vitamin A-therapy, invalidating the use of such rats for assay tests by the smear method.

8. The usefulness of the vaginal cornification test as a method of bio-assay, and as a criterion of mild levels of vitamin A-deficiency, is discussed.

LITERATURE CITED

ABERLE, S. B. D. 1933 Continual cornified vaginal cells as an index of avitaminosis-A in rats. J. Nutrition, vol. 6, pp. 1–10.

———— 1933 a The interrelation of a gonotropic hormone and vitamin A. Am. J. Physiol., vol. 106, pp. 267–272.

BAUMANN, C. A., AND H. STEENBOCK 1932 The vaginal smear method of determining vitamin A. Science, vol. 76, pp. 417–420.

COWARD, K. H. 1929 The influence of vitamin A deficiency on the oestrous cycle of the rat. J. Physiol., vol. 67, pp. 26–32.

COWARD, K. H., B. G. E. MORGAN AND F. J. DYER 1930 The influence of vitamin A deficiency on the oestrous cycle of the rat. II. J. Physiol., vol. 69, pp. 349–352.

EVANS, H. M. 1928 The effects of inadequate vitamin A on the sexual physiology of the female. J. Biol. Chem., vol. 77, pp. 651–654.

EVANS, H. M., AND K. S. BISHOP 1922 On an invariable and characteristic disturbance of reproductive function in animals reared on a diet poor in fat soluble vitamine A. Anat. Rec., vol. 23, p. 17.

HOHLWEG, W., AND M. DOHRN 1930 Kolpokeratose, ein Test für Vitamin A. Ztschr. f. d. ges. exper. Med., Bd. 71, S. 762–767.

LONG, J. A., AND H. M. EVANS 1922 The oestrous cycle in the rat and its associated phenomena. Memoirs Univ. California, vol. 6.

MACY, I. G., J. OUTHOUSE, M. L. LONG AND A. GRAHAM 1927 Human milk studies. I. Technique employed in vitamin studies. J. Biol. Chem., vol. 73, pp. 153–174.

MASON, K. E. 1933 Differences in testis injury and repair after vitamin A-deficiency, vitamin E-deficiency, and inanition. Am. J. Anat., vol. 52, pp. 153–239.

———— 1935 Foetal death, prolonged gestation and difficult parturition in the rat as a result of vitamin A-deficiency. (In press.)

MASON, K. E., AND E. T. ELLISON 1934 Vaginal cornification as a criterion of vitamin A-deficiency in the rat. Anat. Rec., vol. 58, supplement, p. 80 (abstract 179).

———— 1935 The demonstration of oestrus in the vitamin A-deficient rat by supravital study of the vaginal smears. J. Nutrition, vol. 10, p. 1.

MASON, K. E., AND J. M. WOLFE 1935 Relation of castration to vitamin A-deficiency in the rat. J. Nutrition, vol. 9, pp. 725–734.

PARKES, A. S., AND J. C. DRUMMOND 1926 The effects of fat-soluble vitamin A deficiency on reproduction in the rat. Brit. J. Exp. Biol., vol. 3, pp. 251–273.

SEIFRIED, O. 1930 Studies on A-avitaminosis in chickens. I. Lesions of the respiratory tract and their relation to some infectious diseases. J. Exp. Med., vol. 52, pp. 519–531.

THATCHER, H. S., AND B. SURE 1932 Avitaminosis. III. Pathologic changes in tissues of the albino rat during early stages of vitamin A deficiency. Arch. Pathol., vol. 13, pp. 756–765.

WOLBACH, S. B., AND P. R. HOWE 1925 Tissue changes following deprivation of fat-soluble A vitamin. J. Exp. Med., vol. 42, pp. 753–777.

———— 1933 Epithelial repair in recovery from vitamin A deficiency. Ibid., vol. 57, pp. 511–526.

PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures are photographs of vaginal smears of the rat. × 100.

Stained in haematoxylin and eosin.

1 Normal dioestrus. Considerable mucus with many leucocytes and a few nucleated epithelial cells.

2 Early cornification during dioestrus. A few large cornified cells intermingled with leucocytes and large, swollen, nucleated epithelial cells.

3 Early cornification during dioestrus. Approximately equal numbers of completely cornified cells and nucleated cells, some of which exhibit early stages of cornification. Mucus thin and watery.

4 Early cornification during transition from late dioestrus to early prooestrus. Shows many large, swollen nucleated epithelial cells with faintly acidophilic or faintly basophilic nuclei surrounded by a ring of faint cytoplasm. Few leucocytes are present. The mucus is becoming thin and watery. The following day the smear resembled that of figure 9.

5 Normal prooestrus, showing large basophilic nucleated epithelial cells.

6 Normal prooestrus (late), showing smaller nucleated epithelial cells with pycnotic nuclei and faintly acidophilic cytoplasm, as frequently observed during the transition period between prooestrus and oestrus.

7 Normal oestrus, showing the large discrete, flaky cornified cells typical of this stage.

8 Normal metoestrus (late), showing large numbers of basophilic nucleated epithelial cells and leucocytes, together with numbers of pale cornified elements persisting from the preceding stage of oestrus.

9 Atypical prooestrus characteristic of early stages of cornification. The large basophilic nucleated epithelial cells typical of this stage (fig. 5) have undergone extensive swelling and cornification. Pycnotic nuclei are visible in many cells.

10 Moderate cornification appearing during dioestrus. The majority of nucleated epithelial cells show varying degrees of cornification. Abundance of leucocytes, but no mucus.

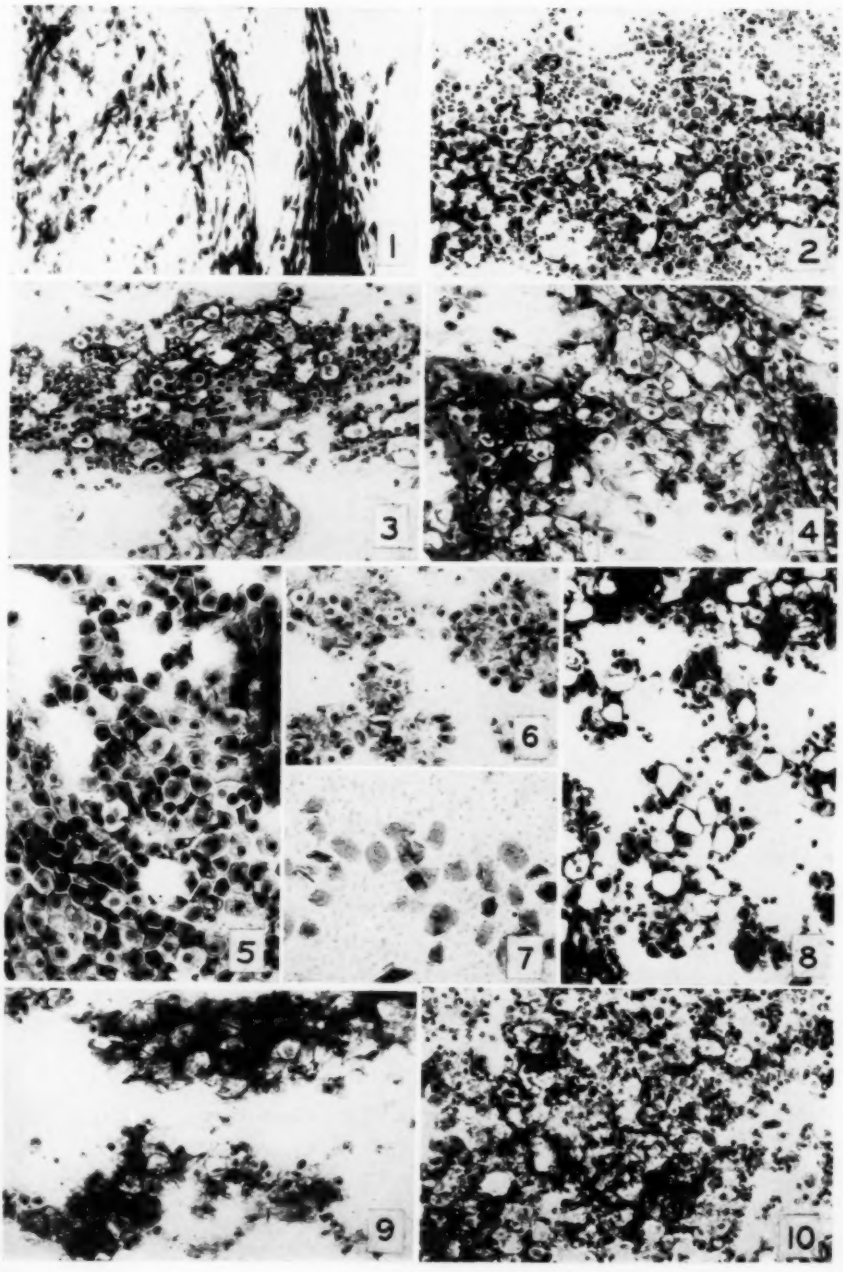
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PLATE 2

EXPLANATION OF FIGURES

Figures 11 to 15, 17 and 18 are photographs of vaginal smears of the rat. × 100.

Figures 16, 19 and 20 are photographs of sections of the vagina of the rat. × 100.

Stained in hematoxylin and eosin.

11 Advanced cornification. Large numbers of cornified cells and leucocytes; very few nucleated epithelial cells.

12 Advanced cornification. Similar to figure 11, except for larger proportion of cornified epithelial cells.

13 Extreme cornification. Only a few leucocytes and extruded nuclei of degenerated nucleated epithelial cells are interspersed among the large completely cornified cells.

14 Extreme cornification. Representing stage of complete cornification. Resembles smear of normal oestrus (fig. 7), except for the extreme paleness of the cornified cells.

15 Vaginal smear on seventeenth day of pregnancy, from rat receiving one-fifth drop of cod liver oil, daily. This level of therapy was adequate to repair the previous vaginal cornification, as demonstrated by the presence of the large mucified epithelial cells which are identical with those commonly observed in normal rats during the second half of pregnancy.

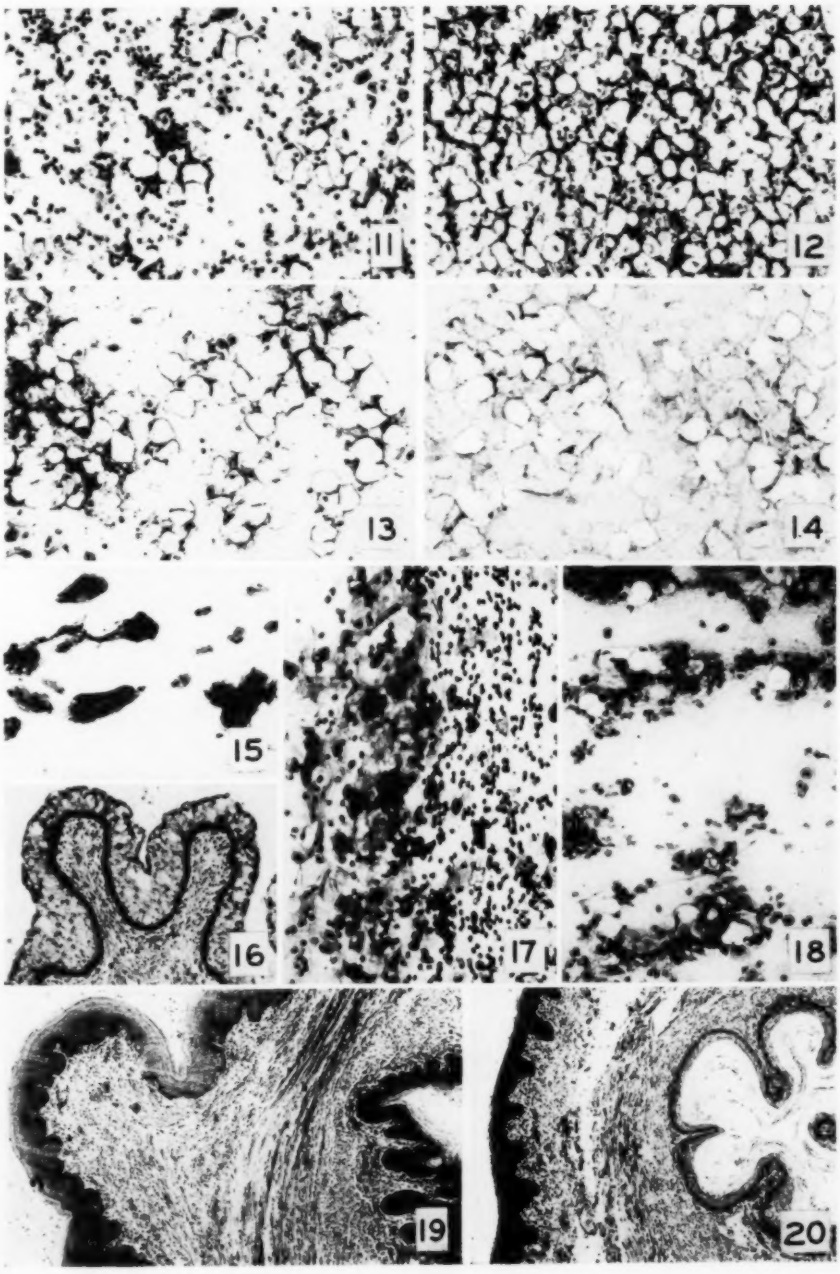
16 Showing the tall mucified type of vaginal epithelium of the normal rat at the end of pregnancy.

17 Vaginal smear on fifteenth day of pregnancy, from rat receiving subminimal level of A-therapy (one-tenth drop of cod liver oil, daily). Such smears are frequent when mild, chronic deficiency of A exists during pregnancy. The deeply stained elements are mucified nucleated epithelial cells similar to those of figure 15. The grayish masses represent many nucleated epithelial cells undergoing all degrees of cornification. A thin, watery mucus is usually present in such smears.

18 Vaginal smear of castrate rat which, after 20 days of continuous cornification, received daily administration of one-fifth drop of cod liver oil for 43 days, and of 2 drops of cod liver oil for 32 more days. Cornified cells continued to persist in the smear. This smear was taken on the seventy-fifth day of therapy.

19 Thick cornified stratified epithelium of vagina (left) and of urethra (right), from A-deficient rat at the end of pregnancy, during which cornification of vaginal smears was complete and continuous.

20 Vaginal epithelium (left) and urethral epithelium (right) from rat which had shown severe vaginal cornification for 18 days after which one-tenth drop of cod liver oil was administered, daily, during the 4 days preceding autopsy. Compare with figure 19, representative of the condition prior to therapy, and note the marked reduction in thickness of both epithelia, due to sloughing of the superficial layers of cornified cells.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PROCEEDINGS OF THE SECOND ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

MINUTES OF MEETING OF AMERICAN INSTITUTE OF NUTRITION, DETROIT, APRIL 10, 1935

The second annual meeting of the American Institute of Nutrition was held in Detroit at the Masonic Temple on April 10, 1935, according to printed program sent to all members. Seventy-six members and 203 guests registered and approximately 400 to 500 people were in attendance during the entire program.

President John R. Murlin presided at the morning scientific session and business meeting. Vice-President E. F. DuBois presided during a part of the afternoon scientific session. The meeting started promptly at 9.30 A.M. and for the most part each speaker was thoughtful enough to confine his comments rigidly to the time allotted to the presentation of his paper thus making it possible to start the business meeting at 11.30 A.M. according to schedule and to close promptly at 1.00 P.M. The second scientific program was conducted in the same expeditious and satisfactory manner. All twenty papers listed on the program were presented.

The business meeting was called to order by President Murlin. The reading of the minutes of the preceding meeting was dispensed with since they had been published in The Journal of Nutrition and could be read by all. The president announced that any comments on the minutes as published could be sent in writing to the secretary.

The report of the Treasurer was then read and accepted. Harold B. Pierce and Harold E. Himwich were asked to serve as auditors. After examination of the treasurer's report they declared it correct.

The annual assessment for 1935–1936 was fixed at $1.00 by unanimous vote of the membership.

The committee appointed to consider changes in the constitution[1] of the American Institute of Nutrition submitted their report. After much discussion of the various items the following changes in the by-laws were adopted (the changes are italicized):

Article I. Membership. Section I. *Membership:*
There shall be two classes of members, members and emeritus members. The number of members shall be limited to 250; exclusive of emeritus members.
Section II. Eligibility for membership: *Members.*—Qualified investigators who have independently conducted and published meritorius original investigations in some phase of the chemistry or physiology of nutrition *and who have shown a professional interest in nutrition for at least 5 years* shall be eligible for membership in the society. *Emeritus members.—Members in good standing who have reached the age of 65 years shall become emeritus members. A member in good standing and for sufficient reason may by vote of the council be made an emeritus member. Emeritus members shall be entitled to vote but not hold office.*
(Change the designation of Sections 3 and 4 to 4 and 5 respectively.)
Article VI. Dues: *The dues shall be the annual cost of subscription to The Journal of Nutrition for members plus an* annual assessment *which* shall be determined by majority vote at the annual meetings, upon recommendation of the council, and shall be due within a month after the annual meeting. *Emeritus members are not required to pay assessments nor to subscribe to The Journal of Nutrition.*
(NOTE: The dues will be $4.00 plus the assessment for those who have already subscribed to the journal for the year 1935. This will place the journal year from July 1st to June 30th. Thereafter the dues will be $8.00 plus the assessment. For members who do not now subscribe to the journal the dues will be $8.00 plus the assessment for the year 1935–1936.)
Article VII. Section 2. (Beginning with the subsection lettered 'A.') Subsection A. *The editorial board shall consist of twelve regular members and a managing editor.* B and C (the present subsections now lettered A and B) the managing editor shall be chosen by the editorial board to serve a period of 5 years. *He shall be eligible for re-election.*

The next item of business was the election of officers. Franklin C. Bing, Ray G. Daggs and Walter C. Russell were asked to act as tellers for this election.

The nominating committee for 1935, composed of E. B. Forbes, Henry A. Mattill (appointed to replace A. W. Rowe,

[1] Original Constitution—see J. Nutrition, vol. 7, no. 5 and supplement, 1934.

deceased), H. S. Mitchell, W. S. Sperry and Harry Steenbock, chairman, proposed the following nominees:

Officers		*Editorial Board* (4 to be elected)
President	J. R. Murlin	G. R. Cowgill
Vice-President	E. F. DuBois	H. B. Lewis
	F. C. Koch	I. G. Macy
Secretary	I. G. Macy	E. M. Nelson
Treasurer	W. M. Boothby	L. S. Palmer
	G. R. Cowgill	W. C. Rose
Councilor	L. A. Maynard	A. T. Shohl
	G. E. Cullen	A. H. Smith

Doctor Steenbock, in discussing the report, stated that the nominating committee had a considerable number of ballots cast in arriving at its list of nominees. The committee appreciated that there were certain questions of precedence which might be involved and was exceedingly careful in arriving at its conclusion. Nevertheless, it saw fit to nominate Doctor Murlin as president in face of his protest against the possible establishment of a precedent of re-electing officers from year to year because it wanted to express in some small measure appreciation for the excellent work he has done for the society. In the case of the secretary, the committee felt that on account of the familiarity of the present secretary with the work involved it would be to the credit and advantage of the society to have her re-elected. Although the president called for nominations from the floor it was moved and seconded that the nominations be closed. Furthermore, it was moved and seconded that the secretary be instructed to cast a unanimous ballot for Doctor Murlin as president and the president in turn was asked to cast a unanimous ballot for Icie G. Macy as secretary. The tellers reported the following election of officers by ballot:

a) Members of Council:
E. F. DuBois, Vice-President
G. R. Cowgill, Treasurer
L. A. Maynard

b) Editorial Board:
W. C. Rose
A. H. Smith
G. R. Cowgill
H. B. Lewis

In discussing the new amendment on dues Paul E. Howe suggested that it be a matter of choice if a member takes one

or two volumes of The Journal of Nutrition at a time. It was also decided that the society should collect the dues (including the annual assessment and the journal fee) and pay to the journal.

The opinion was expressed that it was quite unfair for a member to subscribe to the journal and then turn it over to the library in an institution. The society loses because members pay $4.00 per volume and non-members $5.00.

The council which had met at the Hotel Statler on Tuesday evening, April 9th, reported the following names as qualified for membership in the American Institute of Nutrition:

Leah Ascham
James Harold Austin
Joseph H. Axtmayer
Charles E. Bills
L. Jean Bogert
Alfred Bosworth
W. W. Braman
Margaret Hessler Brookes
Thomas B. Cooley
Gerald J. Cox
Robert Gibson
Estelle E. Hawley
V. G. Heller
C. A. Hoppert
Percy R. Howe
Rebecca Hubbell
J. F. McClendon
E. W. McHenry
Ade T. Milhorat
R. C. Miller
Elsa R. Orent
Herbert Pollack
Myra Sampson
Frederick H. Scharles
Philip A. Shaffer
Henry J. Spencer
F. W. Sunderman
Dee Tourtellotte
Harry M. Vars
James Waddell
Bruce Webster

It was moved by Paul E. Howe and seconded by E. F. DuBois that the candidates approved by the council be elected to membership in the American Institute of Nutrition. The motion was carried unanimously.

The chairman announced the resignation of Robert C. Moulton. E. B. Forbes moved that Doctor Moulton be continued as an emeritus member. This was seconded by E. F. DuBois. The motion carried.

In accordance with the by-laws the chairman appointed the following nominating committee for 1936: Henry C. Sherman, chairman, R. Adams Dutcher, Fred A. Hitchcock, Charles G. King and Helen T. Parsons.

The chairman announced that the council recommends a continuance of the policy for the present at least, of holding the meeting of the American Institute of Nutrition one day in advance of the Federation meeting. There seems to be no objection to this arrangement.

The noon luncheon was attended by 141 members and guests.

R. M. Bethke moved that the following resolution be recorded in the minutes:

That the secretary be instructed to write the Convention Bureau, the Masonic Order and the Local Committee and express the thanks and appreciation of the American Institute of Nutrition for the splendid accommodations provided for the meetings and all other arrangements that added to the comfort and pleasure of the members and their guests.

At the closing of the meeting the president announced the untimely death of Allen Winter Rowe of Boston during the past year. The members stood for a moment in silence in token of respect for Doctor Rowe.

Icie G. Macy,
Secretary.

PROGRAM OF PAPERS PRESENTED

ABSTRACTS

Experiments with a new type of antirachitic substance. Lester Yoder, B. H. Thomas and Malcolm Lyons, Iowa Agricultural Experiment Station, Ames Iowa.

Recently Yoder[1] reported that an antirachitic derivative, namely, cholesterilene sulfonic acid, can be prepared from cholesterol without resorting to irradiation. We have found in working with this derivative and certain of its salts that their effects on intestinal reaction, iron reduction and calcification are very similar to those of viosterol.

The calcium salt of cholesterilene sulfonic acid has been repeatedly prepared and has been found to possess the same constant composition and antirachitic potency. Sulfonic acids prepared in the same manner from a variety of synthetic alcohols have failed to exhibit any antirachitic activity.

In view of the wide differences in the efficacy of cod liver oil and viosterol as antirachitics for chicks, calcium cholesterilene sulfonate was compared with these commonly employed supplements. Two such tests have demonstrated that chicks are able to utilize calcium cholesterilene sulfonate more efficiently than cod liver oil and much more so than viosterol. For example, the tibiae of chicks fed rations containing 2 rat units of cod liver oil or 50 rat units (Steenbock) of viosterol per 100 gm. of ration contained practically the same percentage of ash as those of the negative control group; namely, 28.7 per cent. However, chicks similarly fed rations containing 2 and 50 rat units (Steenbock) of calcium cholesterilene sulfonate yielded bones which ashed practically the same as the positive control group; namely 47.3 per cent.

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[1] Lester Yoder, Science, vol. 80, p. 385, 1934.

Variability of vitamin D response with temperature of environment. D. Tourtellotte (introduced by M. S. Fine) and W. Bacon (by invitation), Research Laboratories, General Foods Corporation, Battle Creek, Michigan.

The routine use of a standardized cod liver oil as a control for vitamin D assays during a 2-year period has shown the variability in vitamin D response with temperature of environment and indicates the necessity for air-conditioned animal quarters where precise vitamin D assays are to be made. Although some nutrition laboratories have such air-conditioned rooms, no published data showing the variability in assay results due particularly to uncontrolled summer temperatures have appeared.

The vitamin D estimations were made by the Bills 5-day line test modification which possesses the advantage of speed and reliability over other existing vitamin D assay procedures. Variables due to dietary constituents have been reduced to a minimum by the purchase of a year's supply of grains after careful preliminary tests as to their suitability. Mean temperatures above 80°F. during the rachitogenic and test periods caused an apparent three- to four-fold fluctuation in vitamin D potency of a standard control cod liver oil. The error of the assay was due to an increased severity of rachitogenesis during periods of elevated

temperatures as shown by the development of standard line test rickets in 11 to 14 days as against the specified period of 21 days. Reducing the calcium: phosphorus ratio of the diet compensated for the increased rate of rachitogenesis.

The comparative antirachitic efficiency of irradiated milk, yeast milk and cod liver oil. R. M. Bethke, W. E. Krauss, P. R. Record (by invitation) and O. H. M. Wilder (by invitation), Departments of Animal Industry and Dairy Husbandry, Ohio Agricultural Experiment Station, Wooster.

A comparison was made with chicks of the antirachitic efficiency of irradiated milk, milk produced by the feeding of irradiated yeast to cows, and cod liver oil, on a rat unit basis.

The dried whole milks were first assayed for vitamin D with rats and then compared on the rat unit basis with chicks. Equivalent amounts of milk solids, either in the form of dried skim milk or dried whole cheek milk, were included in the negative and positive control chick rations. Ash analyses of the tibiae were made at the end of the fifth or sixth week.

The data showed that it required more than ten times as many rat units of vitamin D in the form of yeast milk than in the form of irradiated milk to produce the same degree of calcification. Vitamin D from irradiated milk and cod liver oil were equally efficient.

The same milks (irradiated and yeast) were found to be equally efficient for the cure of rickets in infants by Gerstenberger, Horesh and Van Horn, when fed on an equivalent rat unit basis. These observations indicate that the infant and chick vary greatly in their utilization of vitamin D from different sources.

The antirachitic efficiency of irradiated milk, yeast milk, cod liver oil, and irradiated cholesterol. R. W. Haman (by invitation) and H. Steenbock, Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.

The primary object of our experiments was to compare the antirachitic efficiency of a unit of vitamin D from irradiated milk with that from so-called yeast milk produced by the feeding of irradiated yeast to cows. In line with previous work the milks were standardized with rats and the comparison of efficiency was made with baby chicks. The milks in the first experiments were fed incorporated in a rachitogenic ration. Later butter fat from the milks was used instead. Bone ash analyses were made after 5 weeks.

The data obtained revealed that for the chicken a unit of vitamin D from irradiated milk was approximately ten to fifteen times as efficient as a unit from yeast milk. Irradiated milk proved to be as efficient as cod liver oil. Furthermore our data confirmed the observation of Dr. James Waddell that irradiated cholesterol had the same antirachitic efficiency as cod liver oil.

In view of our data it is evident that there is an urgent need for correlative studies with infants. So far as data with infants are available in the literature, it is evident that the difference in efficiency of cod liver oil and irradiated milk as compared with yeast milk is not the same as we found it to be with chickens.

The healing of rickets in rats on a diet containing negligible amounts of calcium and vitamin D. James H. Jones and B. N. E. Cohn (by invitation), Departments of Physiological Chemistry and Orthopedic Surgery, School of Medicine, University of Pennsylvania, Philadelphia.

Young rachitic rats, which were transferred to a synthetic diet of normal phosphorus content but containing at most only traces of either calcium or vitamin D, showed a marked decrease in food consumption and developed tetany within 18 to 48 hours. After 48 hours the animals were divided into three groups: group I, sacrificed; group II, continued on synthetic diet; group III, given irradiated ergosterol. Serum calcium and phosphorus of group I were about 6 and 10 mg., respectively, and the 'line test' indicated slight healing. The food consumption of groups II and III increased after 48 hours and tetany disappeared. Serum calcium increased while phosphorus remained high. After a few weeks both groups of animals showed complete healing of the rickets. The shafts of the long bones were very fragile with numerous spontaneous fractures. The absolute amount of ash in the femora of the animals of groups II and III appeared to be slightly more than in those of group I. Since healing of the rachitic lesions and the possible slight increase in femur ash occurred on a diet containing negligible amounts of calcium the bone salts must have been transferred from calcified portions of the skeleton to the more rachitic parts. Furthermore, as healing took place in the animals not receiving viosterol apparently vitamin D is not essential for this metastasis.

The influence of some commonly used salt mixtures upon growth and bone development of the albino rat.[1] Lafayette B. Mendel, Rebecca B. Hubbell (by invitation) and Alfred J. Wakeman (by invitation), Biochemical Laboratory of the Connecticut Agricultural Experiment Station, New Haven.

In order to devise a salt mixture suitable for use with rapidly growing rats, a study was made of four well-known preparations. Male rats in groups of ten were placed at weaning on diets of purified foods in which the salt mixture and the level at which it was fed were the variables.

Comparisons of the rate of growth from 60 to 200 gm., and of the ash of the dry, fat free femurs showed that the mixtures are not interchangeable at any of the six levels studied. Rats given 4 per cent Osborne-Mendel or McCollum salts showed the same daily gain (5.2 gm.), but the bone ash was quite different, being 60.8 and 57.9 per cent, respectively. With 2 per cent Steenbock salts, the gain was 4.0 gm. and the bone ash 50.1 per cent, whereas with Osborne-Mendel salts, the animals gained 5.8 gm. per day and attained a higher bone ash (51.8 per cent) in spite of the more rapid growth.

If to 1 per cent of any of the four mixtures enough $CaCO_3$ was added to raise the calcium to the level in 5 per cent Osborne-Mendel salts growth was rapid in all cases (5.0 to 5.8 gm. daily) and calcification of the femurs occurred to about the same degree (57.4 to 58.9 per cent).

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

The influence on iron assimilation of some of the elements in groups 1 and 2 of the periodic system. Seymour W. Kletzien, State Institute for the study of Malignant Disease, Buffalo, New York.

In the past year evidence has been presented by the author showing the definitely inhibitive effect on iron assimilation of calcium carbonate added to an otherwise complete diet. These studies have been extended since the presentation of the initial report to include other calcium salts as well as the carbonates of sodium, potassium, beryllium, strontium, and barium. The data reveal that the role of these elements in iron assimilation is distinct, if not unique, and that they fall according to their ability to facilitate or inhibit iron assimilation into either group 1 or 2 of the periodic system.

The significance of this work aside from its more abstract or purely scientific aspects lies in its direct application to our present day nutrition problems among which the studies in reproduction, anemia, and respiratory disease figure so conspicuously. Further, it appears to throw light on those problems relating to iron and beryllium injections in experimental tuberculosis, and lastly, it affords an apparent answer as to why we are born rich in iron and poor in calcium.

Indispensability of zinc in the nutrition of the rat. Frank E. Stirn (by invitation), C. A. Elvehjem and E. B. Hart, Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.

Data are presented substantiating our former work which demonstrated that zinc was essential in the nutrition of the rat. We had shown that zinc was required for normal growth. However, about the same time Newell and McCollum reported no differences in animals on zinc low and zinc fortified diets with the exception of a variability in the animal's zinc content. In continuing our work the ration was further improved by lowering its zinc content and increasing its growth promoting properties by supplying the water soluble vitamins from milk serum. On this ration the animals which did not receive zinc consistently showed marked retardation of growth as compared to litter mates on the same ration to which zinc had been added. No specific pathological changes were exhibited by the animals on the zinc low ration except that they became hyper-irritable and developed an abnormal fur coat. That the ration was complete in every respect, with the exception of zinc, was shown by the fact that the animals which received this ration with added zinc were apparently normal. Food intakes showed that the zinc low animals were less efficient converters of ration into body weight and also that they consumed appreciably less ration. This work demonstrates conclusively that zinc is an essential element for the normal nutrition of the rat.

The relation of selenium to a toxicosis produced by ingestion of wheat. E. M. Nelson and D. Breese Jones, Protein and Nutrition Division, Bureau of Chemistry and Soils, United States Department of Agriculture.

Four years ago the attention of Dr. Henry G. Knight, chief of the Bureau of Chemistry and Soils, was directed to the fact that a disease of obscure origin occurred in farm animals, and furthermore, that wheat and other grains grown in the same locality where the disease occurred produced toxic symptoms when fed to laboratory animals. Extensive research in the U. S. Department of Agriculture

has established that the toxic nature of such wheat is due to the presence of selenium. Experimental evidence is offered showing that this toxic selenium is associated with certain proteins.

Utilization of inulin by the white rat. Howard B. Lewis and Adelaida Bendana (by invitation), Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor.

The problem of the utilization of inulin was approached through a study of the growth curves of young white rats on a diet adequate except in calorific value. Control animals 60 to 70 gm. in weight, on a diet supplying approximately 12 cal. daily, with either fructose or sucrose as a source of carbohydrate, showed growth curves which were essentially flat. Animals on the basal diet plus 1.5 gm. of inulin (chicory) daily showed much better growth than the controls. However, rats given the basal diet plus 1.5 gm. of either sucrose or fructose daily showed a markedly superior growth as compared with the rats fed inulin. Thus, although inulin may be utilized to some extent, its nutritive value is not comparable to that of either fructose or sucrose.

In a number of experiments in which inulin entirely replaced either sucrose or fructose, all except four rats in one series died within 15 days. Death was probably due to marked distention of the large intestines, with changes in pressure relations caused by intestinal fermentation of the inulin.

An attempt was also made to determine whether any difference exists between inulins of different botanical origins by following growth curves of rats on the basal diet plus 1.5 gm. daily of either dahlia or chicory inulin. No evidence of a difference in the nutritive value was obtained.

Regression as a tool in the biological assay of vitamin A. Pearl P. Swanson and Gladys H. Timson (by invitation), Nutrition Laboratory of the Foods and Nutrition Department, Iowa State College, Ames.

Within limits, gain in weight of depleted rats is a linear function of the quantity of vitamin A fed. The proportionality between dose and response holds after gains induced by graded levels of vitamin feeding have been translated into international units by means of a 'curve of response' fitted by the method of least squares.

Significant correlations have been found between the increase in body weight during the test period and the age and weight of the animal at the end of the depletion period, the quantity of food consumed during this interval, and the length of the depletion period.

By means of a regression equation involving the above factors and calculated from data on 577 female rats, the mean response of groups of rats to 0.5 International unit has been successfully estimated. Information concerning expected behavior of individual A-depleted rats ready for supplementary feeding may be gained by the same regression. Those animals whose response can thus be estimated as erratic are eliminated at this stage, whereby, increased reliability of results is obtained with a decreased number of animals.

The influence of vitamin C level upon resistance to diphtheria toxin. C. G. King and M. L. Menten (by invitation), University of Pittsburgh.

Guinea pigs under controlled conditions and receiving definite daily quantities of vitamin C at abundant, protective, and sub-protective levels were given subcutaneous injections of standardized diphtheria toxin in 0.1, 0.3, 0.5, and 1.0 M.L.D. quantities. The survival time was shortened about 50 per cent and loss in body weight was more severe when the animals were partially depleted of their vitamin C reserves, without showing external signs of scurvy. Contrasts in degree of hemorrhage and necrosis at the sites of injection were more marked than weight and survival differences. It is evident from the study that there is a wide zone of vitamin C deficiency, without the appearance of scurvy, where physiological processes are subnormal and the animal is more sensitive to injury from bacterial toxin. Histological examination of the tissues from a large number of experimental animals has confirmed the differences in degree of injury observed macroscopically, and has also indicated a close relationship to certain human pathological conditions of particular interest is the relationship to arteriosclerosis.

The effects of the plane of protein intake on the utilization of food energy and protein. E. B. Forbes, R. W. Swift, Alex Black (by invitation) and O. J. Kahlenberg (by invitation), Institute of Animal Nutrition, Pennsylvania State College.

The effects of four planes of protein intake were studied in a 10 weeks growth, metabolism and body analysis experiment on forty-eight albino rats.

The diets contained 10, 15, 20 and 25 per cent protein, and were made equicaloric by compensatory change in carbohydrate and fat.

The rats were selected as twelve groups of four individuals, each group being of one sex and of the same litter, the rats in each such group receiving the same energy but different protein intake.

Growth increased with increase in protein to 20 per cent, but the 25 per cent group grew essentially the same as did the 20 per cent group.

With increased protein but constant energy intake the digestibility of the protein increased slightly, and linearly; urinary nitrogen increased at an increasing rate, and protein of body gain increased at a decreasing rate.

With increased protein but constant energy intake the feces energy decreased as the urinary energy increased, the metabolizable energy therefore being practically constant. The energy of the body gain and of the protein gain increased materially but of the body fat there was not significant gain.

With increased protein but constant energy intake the heat production diminished materially, which is considered to be due to the removal from the circulation of heat-stimulating nutrients, as results of improved nutritive balance and increased synthesis of body substance.

The variability of the heat production of normal human subjects. Joseph Berkson (by invitation), Halbert L. Dunn (by invitation) and Walter M. Boothby, Section on Biometry and Medical Statistics and Section on Clinical Metabolism, The Mayo Clinic, Rochester, Minnesota.

A statistical analysis of the observations of heat production in relation to age, stature, and body weight has been made in a large series of normal human

subjects (641 males, 776 females). These included series of successive observations made on the same individual (intra-individual) as well as single observations made on successive individuals (inter-individual).

Particular stress is laid in this presentation on a study of the normal variability of heat production both intra-individual and inter-individual. 1) Observations of heat production made on the individual correspond in general to the statistical bell-shaped 'normal curve.' When numerous observations are made on the same subject the concept of random variation is applicable to them. 2) We found that the intra-individual variability accounts for a large part of the variation observed in single observations made on numerous subjects.

Water balance. L. H. Newburgh and Margaret W. Johnston (by invitation), Department of Internal Medicine, University of Michigan, Ann Arbor.

The weights of the following items need to be known in order to deal quantitatively with water balance: Sources: 1) ingesta; 2) endogenous, a) oxidation, b) preformed. Losses: 3) urine; 4) stool; 5) vapor. In order to calculate water of oxidation, preformed water and water vapor, the subject is fed a constant diet of known composition and low in carbohydrate for at least 3 days preceding the period and continued during the study. Under these circumstances, he will metabolize the carbohydrate of the diet. The protein metabolism is calculated from the N excretion. The subject is instructed to adjust the clothing so that he will be comfortable, i.e., he is neither too warm nor too cool.

Since I.W. (vapor) = I.L. (insensible loss of weight)—(CO_2–O_2), the weight of I.W. can be calculated as soon as the fat metabolized is known. This last value may be computed by means of the following equation:

$$F = \frac{\text{I.L.} - (2.12\,\text{CHO} + 10.56\text{N})^1}{3.78}$$

The total CO_2 and O_2 of the metabolic mixture may now be calculated and I.W. derived.

The water of oxidation is calculated from the metabolic mixture and preformed water derived by comparing the diet with the metabolic mixture.

The water of the ingesta, urine and stool, is obtained according to standard procedures.

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[1] Lavietes, P. H.: Journal of Clinical Investigation, 1935, page 57.

The inter-relationship between nitrogen, calcium and phosphorus metabolism of pre-school children. Jean E. Hawks (by invitation), Merle M. Bray (by invitation), Marjorie G. Olsen (by invitation) and Marie Dye, Home Economics Section, Michigan Agricultural Experiment Station, Michigan State College.

During 24 consecutive days, following a 20-day preliminary period, four normal pre-school children received a constant diet containing 3 gm. of protein per kilogram of body weight. For the next 24 days they had the same diet except that the protein was increased to 4 gm. per kilogram. Calcium and phosphorus remained constant. Food, feces and urine were analyzed for nitrogen, calcium and phosphorus.

Values for the fairly constant nitrogen retentions followed intake variations closely on the first diet and on the last 15 days of the second diet, but showed little correlation during the first 9 days following the diet change. On the second diet, nitrogen retentions were higher, calcium retentions slightly lower and phosphorus retentions slightly higher.

The values for nitrogen, calcium and phosphorus retentions, expressed as retention ratios, indicate an inter-relationship between the utilization of these minerals. The increased diet protein caused a greater nitrogen retention which possibly carried with it a slightly greater proportion of phosphorus than the nitrogen retained on the lower protein diet. It also lowered the Ca/P retention ratio suggesting an increased growth of tissue other than bone. It may have limited the amounts of calcium and phosphorus available for bone growth by depositing absorbed phosphorus with nitrogen. Thus it may have altered metabolic processes.

The effect of various dietary principles on milk production. Ray G. Daggs, Department of Vital Economics, University of Rochester, Rochester, N. Y.

The method for the study of lactation in rats, reported by title at the 1934 meeting of the American Institute of Nutrition, is dependent upon two growth constants derived from plotting the logs of the litter weights from the fourth to the seventeenth day of life against time in days. Two straight lines are obtained, the break occurring at the tenth day. By combining the two slope constants a relative lactation index is obtained. The relative lactation value of different diets is shown by these indices.

By using this method the lactation promoting factor, as determined from some 300 experiments, was found to be present in liver, egg, beef round, kidney, alcohol-water extract of liver, diets of higher protein level (liver and casein), water extract of autolysed liver or egg, Witte's peptone, blood fibrin, lactalbumin, cystine and a combination of 1 part cystine, 1 part glycine and 1 part glutamic acid fed with only 12 parts of casein and the other constituents of the basal diet. This last mixture which simulates glutathione gave the best results, although cystine alone as a supplement showed a remarkable effect. It is believed that cystine or glutathione acts in some way as a mammary stimulant.

Two human cases to whom the amino acid mixture was fed showed favorable results.

Continuous nitrogen and mineral balances during pregnancy, puerperium and lactation. Helen A. Hunscher, Frances Cope (by invitation), Helen R. Sternberger (by invitation), Betty Nims Erickson (by invitation) and Icie G. Macy, Children's Fund of Michigan, Detroit, Michigan.

Continuous metabolic balance studies were made from the one-hundred and thirty-fifth day of the fourth pregnancy to the fifty-third day of lactation including the puerperium, on a healthy woman under our constant observation for 6 years.

The diet voluntarily chosen abounded in minerals, protein and vitamins from fruits, vegetables and 2 quarts of milk each containing 150 Steenbock units vitamin D (Vitex). Data on the storage of seven acid-base mineral elements and N are reported for twenty-eight periods of 5 days each during pregnancy and eight periods during lactation. The mean daily food intake during pregnancy, Ca, Mg, Na, K and N were as follows: 3.1, 0.6, 5.0, 6.6 and 19.0 gm. and their

retentions were 0.37, 0.11, 0.56, 1.40 and 3.06 gm., respectively. The ingestion of P, Cl and S amounted to 2.7, 7.7 and 1.5 gm. with resultant daily storage of 0.26, 0.60 and 0.34, respectively. Although the balances were continuous and the ingestion fairly constant the storage fluctuated markedly resulting at term in a large maternal reserve.

During lactation lowered rates of storage and even large losses were encountered as in previous short time balance studies.[1] The average daily balances for Ca, Mg, Na, K and N were — 0.48, — 0.03, + 0.47, + 0.87 and — 0.56 gm. and for P, Cl and S, — 0.37, + 0.30 and — 0.22, respectively

[1] J. Biol. Chem., vol. 86, p. 37 (1930); ibid, p. 59; ibid, vol. 91, p. 675 (1931); ibid, vol. 99, p. 507 (1933).

Cataract in rats fed on galactose. Helen S. Mitchell, Division of Home Economics, Massachusetts State College, Amherst, Mass. Work done at Battle Creek College, Battle Creek, Mich.

A previous publication reported the occurrence of cataractous changes in the eyes of all rats fed on rations containing lactose as the chief source of carbohydrate. Negative results with other carbohydrates tested led to an investigation of galactose as the next logical step. This sugar was fed to young rats at 35 per cent and 25 per cent levels corresponding to the galactose available from the 70 per cent and 50 per cent lactose rations fed in previous experiments.

Four rats on the 35 per cent galactose ration developed mature bilateral cataract in 12, 14, 14 and 37 days, respectively (average 19 days), whereas those on the 25 per cent galactose ration were somewhat more delayed. The average time for the development of mature bilateral cataract in forty-nine rats fed the 70 per cent lactose ration was 10 weeks, approximately four times as long.

Galactosuria was more severe on galactose than on lactose rations. The increased calcium content of cataractous eyes was apparent in a shorter time. Galactose rations caused no diarrhea—a consistent finding with lactose feeding.

Rapid and complete absorption of galactose from the intestine, a slow glycogenesis and low kidney threshold reported by previous workers seem to be indicated.

Degenerative changes in the lens capsular membrane due to galactose injury, followed by infiltration of inorganic ions might be sufficient to cause the precipitation of proteins of the crystalline lens.

Role of the liver and pancreas in the dextrose tolerance curve. Samuel Soskin, The Metabolic Laboratory, Department of Physiology, Michael Reese Hospital, and Department of Physiology, The University of Chicago.

It has been shown that the presence of the pancreas is not essential to the normal dextrose tolerance curve, while the presence of the normal liver is essential. One of the major factors in determining the occurrence of the normal curve, is a homeostatic reaction of the liver, in which it decreases its supply of sugar to the blood in response to the influx of exogenous sugar.

These results and conclusions yield a new basis for interpreting those metabolic disturbances in which abnormal dextrose tolerance curves occur. The application of these facts to toxemia, diabetes, starvation, high carbohydrate therapy and 'insulin resistance' is briefly discussed.